Document Title

Summary of the ecotoxicological studies
Prothioconazole + Spiroxamine EC 460 (160+300 g/L)

Data Requirement(s)

Regulation (EC) No 1107/2009 & Regulation (EC) No 284/2013

Document MCP

Section 10: Ecotoxicological studies

According to the Guidance Document SANCO/10181/2013 for applicants on preparing dossiers for the approval of a chemical active substance

Date

2021-03-31

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ERM

On behalf of Bayer AG

Crop Science Division

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Version history

Date [yyyy-mm-dd]	Data points containing amendments or additions[1] and brief description	Document identifier and version number

[1] It is suggested that applicants adopt a similar approach to showing revisions and version history as outlined in SANCO/10180/2013 Chapter 4, 'How to revise an Assessment Report'

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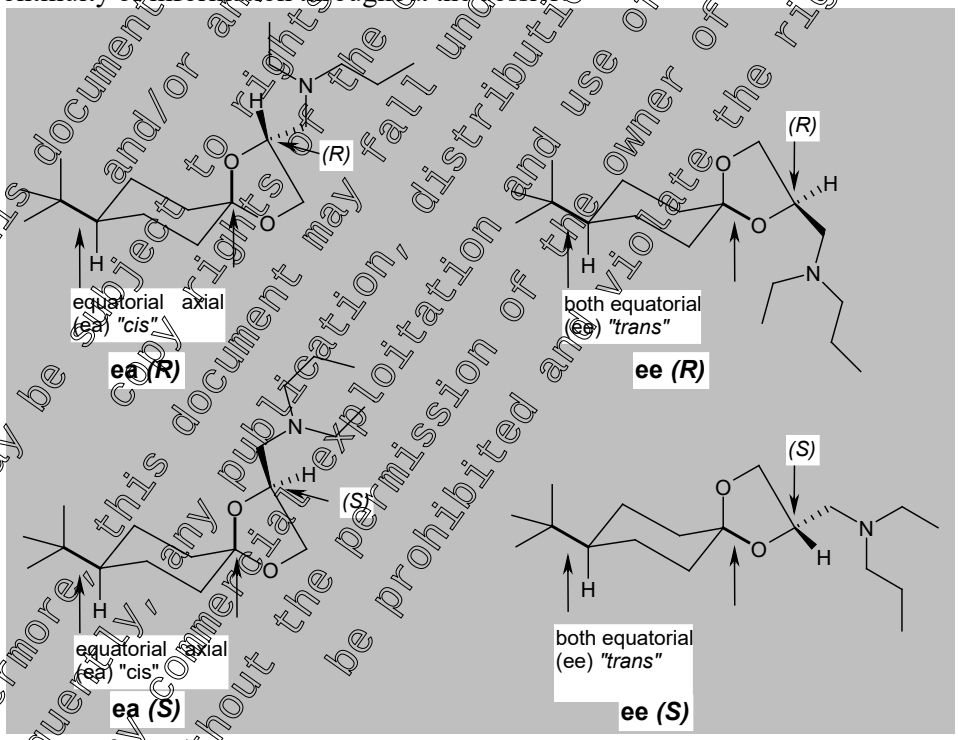
CP 10 ECOTOXICOLOGICAL STUDIES ON THE PLANT PROTECTION PRODUCT

Spiroxamine was included in Annex I to Council Directive 91/414/EEC in 1999 (Directive 1999/73/EC, Entry into Force on 1 September 1999). This Supplementary Dossier contains data which were not submitted at the time of the Annex I inclusion and first renewal of Spiroxamine under Council Directive 91/414/EEC and which were therefore not evaluated during the first EU review and first renewal. However, all studies submitted for the first approval and subsequent first renewal of spiroxamine have also been summarised according to current guidance and included in the dossier. Where studies meet relevant validity criteria, new robust study summaries have been provided in the appropriate dossier section. However, where studies do not meet relevant validity criteria and are not considered acceptable, less detailed summaries may have been provided alongside discussions of study deficiencies. All relied upon study reports are submitted in Document K for this second renewal of approval dossier or in Document K for the previous Annex I inclusion and first renewal submissions.

All data which were already submitted by Bayer AG (former Bayer CropScience) for the Annex I inclusion and first renewal under Council Directive 91/414/EEC are contained in the draft Re-Assessment Report (RAR) 2010 and its revised RAR 2017, and are included in the Baseline Dossier provided by Bayer AG.

Spiroxamine consists of four isomers (two diastereomers each with its corresponding two enantiomers which are in a 1:1 ratio) as shown in the schematic below. The A and B nomenclature presented may differ in some historical documentation as a result of a discrepancy in referencing, which is discussed in detail in position paper M-761468-01-1 (see CA 1.7/01). It is recommended that the stereo assignments depicted here, together with the A and B notation should be used exclusively going forward to ensure continuity of information throughout the dossier.



CP 10.1 Effects on birds and other terrestrial vertebrates

CP 10.1.1 Effects on birds

The available avian toxicity data for spiroxamine are summarized in the table below.

Table CP 10.1.1-1 Summary of avian toxicity studies with spiroxamine

Organism	Test item	Test type	Endpoints		Reference
Bobwhite quail (*Colinus virginianus*)	Spiroxamine	Acute oral toxicity	LD_{50} 565 mg a.s./kg bw	EU[1]	M-008095-02-1
Canary (*Serinus canarius*)	Spiroxamine	Acute oral toxicity	LD_{50} 250 - 500 mg a.s./kg bw	EU	M-008100-01-1
Bobwhite quail (*Colinus virginianus*)	Spiroxamine	Short-term dietary toxicity	LC_{50} >5000 mg a.s./kg diet **(LDD_{50} >357 mg a.s./kg bw/day)**	EU	M-008081-02-1
Mallard duck (*Anas platyrhynchos*)	Spiroxamine	Short-term dietary toxicity	LC_{50} 5000 mg a.s./kg diet (LDD_{50} 871 mg a.s./kg bw/day)	EU	M-008047-02-1
Mallard duck (*Anas platyrhynchos*)	Spiroxamine	Short-term dietary toxicity	LC_{50} >312 mg a.s./kg diet (LDD_{50} >81.4 mg a.s./kg bw/day)	EU	M-000872-01-1
Bobwhite quail (*Colinus virginianus*)	Spiroxamine	Reproductive test	NOEC 29.3 mg a.s./kg diet NOEL 2.02 mg a.s./kg bw/day NOAEC 78.6 mg a.s./kg diet **NOAEL 5.40 mg a.s./kg bw/day**	EU	M-007470-03-1
Mallard duck (*Anas platyrhynchos*)	Spiroxamine	Reproductive test	NOEC 78.8 mg a.s./kg diet NOEL 10.6 mg a.s./kg bw/day	EU	M-008186-01-1

EU: previously evaluated as part of the original EU review and listed in EFSA conclusion and DAR
Values in **bold** have been used in the risk assessment

The available avian toxicity data for prothioconazole and prothioconazole-desthio are summarized in the table below.

Table CP 10.1.1-2 Summary of avian toxicity studies with prothioconazole and prothioconazole-desthio

Organism	Test item	Test type	Endpoints		Reference
Bobwhite quail (*Colinus virginianus*)	Prothioconazole	Acute oral toxicity	LD_{50} >2000 mg a.s./kg bw	EU	EFSA Conclusion[1]

Organism	Test item	Test type	Endpoints		Reference
Bobwhite quail (*Colinus virginianus*)	Prothioconazole	Short-term dietary toxicity	LC_{50} >5000 mg a.s./kg diet (**LDD_{50} >1413 mg a.s./kg bw/day**)	EU	
Mallard duck (*Anas platyrhynchos*)	Prothioconazole	Short-term dietary toxicity	LC_{50} ≥5000 mg a.s./kg diet (LDD_{50} >2457 mg a.s./kg bw/day)	EU	
Bobwhite quail (*Colinus virginianus*)	Prothioconazole	Reproductive test	NOEC 1000 mg a.s./kg diet NOEL 86 mg a.s./kg bw/day	EU	
Mallard duck (*Anas platyrhynchos*)	Prothioconazole	Reproductive test	NOEC 700 mg a.s./kg diet **NOEL 78 mg a.s./kg bw/day**	EU	
Bobwhite quail (*Colinus virginianus*)	Prothioconazole-desthio	Acute oral toxicity	LD_{50} >2000 mg/kg bw	EU	
Bobwhite quail (*Colinus virginianus*)	Prothioconazole-desthio	Short-term dietary toxicity	LC_{50} >4090 mg/kg diet (**LDD_{50} >297 mg/kg bw/day**)	EU	
Bobwhite quail (*Colinus virginianus*)	Prothioconazole-desthio	Reproductive test	NOEC 173 mg/kg diet **NOEL 14.8 mg/kg bw/day**	EU	
Mallard duck (*Anas platyrhynchos*)	Prothioconazole-desthio	Reproductive test	NOEC 500 mg/kg diet NOEL 63 mg/kg bw/day	EU	

EU: previously evaluated as part of the original EU review and listed in EFSA conclusion and DAR
[1] EFSA Scientific Report (2007) 106, 1-98. Conclusion on the peer review of prothioconazole
Values in **bold** have been used in the risk assessment

Toxicity endpoints for risk assessment

For the acute risk assessment of spiroxamine the lowest reliable acute LD_{50} value for spiroxamine technical was determined to be 565 mg a.s./kg bw. However, the short term dietary toxicity study with the bobwhite quail determined a lower LDD_{50} of >357 mg a.s./kg bw/day. Thus, the acute risk assessment has been conducted using the more conservative value of >357 mg a.s./kg bw/day.

An ecotoxicologically relevant NOAEL of 5.40 mg a.s./kg bw/day has been set and used in the risk assessment. Justification has been provided below.

The NOEC determined in the reproduction study with bobwhite quail (M-007470-03-1) was 29.3 mg a.s./kg diet (equivalent to 2.02 mg a.s./kg bw/day) and has been based on the statistically significant effects on 14-day survivor body weight at 78.6 mg a.s./kg diet. This NOEC is considered to be very conservative because there was only a 3.7% reduction in body weights, relative to the control, at 78.6 mg a.s./kg diet. Whilst statistically significant, this reduction is not considered to be a true treatment related effect as the reduction is very minor and unlikely to cause an impact at the population level.

It may be a statistical anomaly or intrinsic variability instead of a substance related effect since over the weeks the body weights of 14 day survivors varied considerably. They were statistically reduced in three of the weeks but in two of the weeks they were reduced without statistical significance. In one of the weeks the body weights were equal to the control, but in three of the weeks they were higher than the control. In the last two weeks for example the mean body weights of 14 day old survivors were 34.3 g and 33.7 g, at 78.8 mg a.s./kg diet, while the control chicks weighed 32.3 g in that period. In contrast to these slight and partially contradictory effects, the results at the next highest test concentration (204 mg a.s./kg diet) were consistent and clear. The reduction of body weight of 14 days survivors compared to the control at this dose group amounted to 8.8 %. This also is not a dramatic decline but the average body weights were reduced over the whole exposure period (6 times statistically significant, 3 times without statistically significance). These findings indicate that at this test concentration (204 mg a.s./kg diet) the effects have to be considered treatment related. It is therefore considered that the true LOEC is 204 mg a.s./kg diet and the NOAEC is 78.6 mg a.s./kg diet (equivalent to 5.40 mg a.s./kg bw/day).

To confirm this conclusion, additional statistical analyses of the reproduction data were conducted and presented in report (M-279402-01-1). As part of the analyses, the data were re-evaluated using the mean body weight of all the 14-day old chicks which were produced by each single pair of adults. When the data were assessed in this way a NOEC of 78.6 mg a.s./kg food (5.40 mg a.s./kg bw/day) was determined. The analyses demonstrated how minor the body weight reductions at the 78.6 mg a.s./kg food dose group were in relation to the controls and that the NOEC could legitimately be increased from 29.3 mg a.s./kg diet (equivalent to 2.02 mg a.s./kg bw/day) to 78.6 mg a.s./kg food (5.40 mg a.s./kg bw/day).

Further supporting data has been provided in report M-304594-01-1 in the form of historical control data for 14-day old survivors to demonstrate that the mean body weight of 32.6 g achieved at the 78.6 mg a.s./kg diet dose group was well within the normal deviation of the historical control data from 59 regulatory studies and is not therefore a biologically relevant reduction.

In conclusion, the differences of the chick body weights between the 78.6 mg a.s./kg food dose group and the control were small (3.7%) and the statistical significance of the difference varies according to the various methods used to analyse the data. Taking all of the above information into account it is considered justified to set an ecotoxicologically relevant NOAEL of 5.40 mg a.s./kg bw/day.

According to the outcome of the pesticides peer review meeting on recurring issues in ecotoxicology (EFSA PPR meeting 133, September 2015), an ecotoxicologically relevant endpoint should be set in collaboration with mammalian toxicologists, where required, and should be used in all the steps of the risk assessment. The NOAEL of 5.40 mg a.s./kg bw/day has therefore been used in all tiers of the avian risk assessment for spiroxamine.

For prothioconazole the endpoints that have been presented in the 2007 EFSA Conclusion have been used without further consideration. Discussion over the endpoints for prothioconazole is not considered to be part of the Renewal of Approval for spiroxamine.

No avian acute oral toxicity data are available for Prothioconazole + Spiroxamine EC 460.

Metabolites

Numerous metabolites of spiroxamine are formed in plants following application to crops. In the Toxicology and Residues sections of the dossier the spiroxamine metabolites have been categorised into three distinct groups (Group A, B and C, respectively). Toxicology dara are available for several of these metabolites. Metabolite M13 has been used to represent all Group B metabolites therefore the toxicology data generated using this metabolite is also considered to cover the plant metabolites M35 and M36. Likewise, M28 has been used to represent all Group C metabolites therefore the toxicology data generated using this metabolite is also considered to cover the plant metabolites M29, M30 and M31. It is also noted that Group A metabolites are considered to be covered by parent spiroxamine.

The table below presents each plant metabolite along with the percentage TRR and actual residue value from the crop metabolism studies. Available toxicology data have also been presented as well as an

indication of whether or not each plant metabolite was also found in the animal metabolism studies on laying hen, rat and goat. Finally, an assessment is made regarding the relevance of each plant metabolite to the risk assessment. Only metabolites which were formed in plants at ≥10% TRR are considered to be potentially relevant to the bird and mammal risk assessment.

Note that only metabolites which were found in the crop metabolism studies have been presented below, however M13 and M13-acetate have been included in the table below because there are toxicology data which are relevant to other Group B plant metabolites.

Table CP 10.1.1-3 Assessment of potential exposure of birds to metabolites of spiroxamine formed in plants

Plant Metabolite	Maximum levels of residue in plants	Metabolite found in animal studies?	Mammalian toxicity data available?	Conclusion on relevance for avian risk assessment
Spiroxamine - desethyl (M01) [GROUP A]	**Primary crops** Wheat Forage: 5.1% TRR; 1.11 mg/kg Straw: 2.0% TRR; 0.70 mg/kg Grain: 0.5% TRR; <0.001 mg/kg Grapes 2.1 % TRR; 0.27 mg/kg Banana Pulp: 1.1% TRR; 0.005 mg/kg Peel: 2.7% TRR; 0.18 mg/kg **Rotational crops** Leafy vegetables 12.6% TRR; 0.026 mg/kg Cereals 20.0% TRR; 0.419 mg/kg Root & tuber vegetables 9.3% TRR; 0.083 mg/kg	Not found in goat or rat Found in laying hen (21.3% in liver, 9.3% in muscle, 8.4% in fat and 11.5% in eggs)	No data available.	Metabolite found in primary crops at <10% TRR therefore not considered relevant for risk assessment. Metabolite found >10% TRR in rotational crops but actual residue levels were very low. Metabolite found in hen metabolism study therefore toxicity data and associated assessment for parent considered to cover this metabolite.

Plant Metabolite	Maximum levels of residue in plants	Metabolite found in animal studies?	Mammalian toxicity data available?	Conclusion on relevance for avian risk assessment
Spiroxamine - despropyl (M02) [GROUP A]	**Primary crops** Wheat Forage: 4.6% TRR; 0.49 mg/kg Straw: 4.2% TRR; 3.48 mg/kg Grain: 3.0% TRR; 0.002 mg/kg Grapes 1.5% TRR; 0.20 mg/kg Banana Pulp: 0.5% TRR; 0.002 mg/kg Peel: 2.9% TRR; 0.19 mg/kg **Rotational crops** Leafy vegetables 51.2% TRR; 0.053 mg/kg Cereals 46.6% TRR; 0.190 mg/kg Root & tuber vegetables 21.1% TRR; 0.188 mg/kg	Not found in goat or rat. Found in laying hen (21.7% in liver, 11.3% in muscle, 3.4% in fat and 10.2% in eggs)	No data available.	Metabolite found in primary crops at <10% TRR therefore not considered relevant for risk assessment. Metabolite found <10% TRR in rotational crops but actual residue levels were very low. Metabolite found in hen metabolism study therefore toxicity data and associated assessment for parent considered to cover this metabolite.
Spiroxamine - N-oxide (M03) [GROUP A]	**Primary crops** Wheat Forage: 12.7% TRR; 3.06 mg/kg Straw: 22.0% TRR; 7.68 mg/kg Grain: 17.8% TRR; 0.012 mg/kg Grapes 4.7% TRR; 0.61 mg/kg Banana Pulp: 1.2% TRR; 0.007 mg/kg Peel: 4.9% TRR; 0.23 mg/kg **Rotational crops** Cereals 7.4% TRR; 0.235 mg/kg	Not found in goat or laying hen Found in rat (found in liver at low amounts of 0.1%)	Acute oral rat LD_{50} 707 mg/kg bw 28-day rat oral dietary NOAEL 12.9/13.2 mg/kg bw/day for males/females 90-day rat oral dietary NOAEL 8.8/9.7 mg/kg bw/day for males/females	Metabolite found in wheat at >10% TRR therefore considered relevant for risk assessment. Tox data are available and show that metabolite toxicity in the rat is comparable to that of spiroxamine. It is considered that this can also be extrapolated to birds therefore the avian reproductive risk assessment for spiroxamine covers the risk to this metabolite.

Plant Metabolite	Maximum levels of residue in plants	Metabolite found in animal studies?	Mammalian toxicity data available?	Conclusion on relevance for avian risk assessment
Spiroxamine - N-formyl-desethyl (M04) [GROUP A]	**Primary crops** Wheat Forage: 5.8% TRR; 1.40 mg/kg Straw: 9.7% TRR; 8.06 mg/kg Grain: 6.9% TRR; 0.005 mg/kg Grapes Not found Banana Not found **Rotational crops** Cereals 6.4% TRR; 0.204 mg/kg	Not found in goat, rat or laying hen	No data available.	Metabolite found in primary crops and rotational crops at <10% TRR therefore not considered relevant for risk assessment.
Spiroxamine - hydroxyl (M05) [GROUP A]	**Primary crops** Wheat Forage: 7.1% TRR; 1.71 mg/kg Straw: 5.2% TRR; 4.32 mg/kg Grain: 1.6% TRR; 0.001 mg/kg Grapes 0.3% TRR; 0.04 mg/kg Banana Not found **Rotational crops** Leafy vegetables 17.2% TRR; 0.146 mg/kg Cereals 2.5% TRR; 0.49 mg/kg Root & tuber vegetables 3.6% TRR; 0.032 mg/kg	Not found in goat, rat or laying hen	No data available.	Metabolite found in rotational crops at >10% TRR in leafy vegetables but the actual residues level is very low therefore not considered relevant for risk assessment.
Spiroxamine - hydroxy-despropyl (M09) [GROUP A]	**Primary crops** Wheat Forage: not found Straw: 0.3% TRR; 0.11 mg/kg Grain: not found Grapes Not found Banana Not found	Not found in goat, rat or laying hen	No data available.	Metabolite found in primary crops and rotational crops at <10% TRR therefore not considered relevant for risk assessment.

Plant Metabolite	Maximum levels of residue in plants	Metabolite found in animal studies?	Mammalian toxicity data available?	Conclusion on relevance for avian risk assessment
Spiroxamine – cyclohexanol (M13) [Group B]	**Primary crops** Not found	Not found in goat, rat or laying hen	Acute oral rat LD_{50} 4200 mg/kg bw Acute dermal rabbit LD_{50} >5000 mg/kg bw 28-day rat oral (gavage) NOAEL 50 mg/kg bw/day It was concluded that M13 is less toxic than the parent, spiroxamine in the rat with a *ca.* 9-fold, 2-fold and 8-fold increase in sub-acute, maternal and developmental NOAELs, respectively when compared to the spiroxamine equivalent studies	Metabolite not found in crop metabolism studies therefore not considered relevant for risk assessment. Tox data are available and confirm M13 to be less toxic than parent. M13 data used to represent the toxicity of all Group B metabolites.
Spiroxamine cyclohexanol acetate (M-13 acetate) [Group B]	**Primary crops** Not found	Not found in goat, rat or laying hen	Rat developmental NOAEL maternal toxicity 40 mg/kg bw/day Rat developmental NOAEL 160 mg/kg bw/day Group B metabolites considered to be covered by available data for M13 which confirm that this metabolite is less toxic than spiroxamine.	Metabolite not found in crop metabolism studies therefore not considered relevant to the risk assessment. Tox data are available and suggest lower toxicity than parent spiroxamine.

Plant Metabolite	Maximum levels of residue in plants	Metabolite found in animal studies?	Mammalian toxicity data available?	Conclusion on relevance for avian risk assessment
Spiroxamine-diol (M14) [Group B]	**Primary crops** Banana pulp (12.8% TRR; 0.057 mg/kg- hydrolysis product) Grapes (13.0% TRR; 0.44 mg/kg- hydrolysis product) Spring wheat straw (0.2% TRR; 0.070 mg/kg- hydrolysis product) Spring wheat grain (2.6% TRR; 0.002 mg/kg- hydrolysis product) **Rotational crops** Swiss chard leaves (8.8-13.2% TRR; 0.01 mg/kg- hydrolysis product) Wheat straw (3.5-4% TRR; 0.05 mg/kg- hydrolysis product) Turnip tops (4.4-13.0% TRR; 0.02 mg/kg- hydrolysis product)	Not found in goat, rat or laying hen	No data available.	Metabolite found in plants at >10% TRR but the actual residues level is very low therefore not considered relevant for risk assessment. Toxicity would be covered by parent assessment as M13 data confirm this group of metabolites to be less toxic than parent.
Spiroxamine-ketone (M15) [Group B]	**Primary crops** Grapes (1.3% TRR-hydrolysis product) Spring wheat straw (5.5% TRR-hydrolysis product) Spring wheat grain (4.6% TRR-hydrolysis product)	Not found in goat, rat or laying hen	No data available.	Metabolite found in plants at <10% TRR therefore not considered relevant for risk assessment.
Spiroxamine-hydroxy-ketone (M16) [Group B]	**Primary crops** Grapes (0.5% TRR-hydrolysis product) Spring wheat straw (1.0% TRR-hydrolysis product) Spring wheat grain (7.6% TRR-hydrolysis product) **Rotational crops** Swiss chard leaves (15.6-29.3% TRR; 0.04 mg/kg- hydrolysis product) Wheat straw (8.9-11.6% TRR; 0.15 mg/kg- hydrolysis product) Turnip tops (11.7-37.3% TRR; 0.17 mg/kg- hydrolysis product)	Not found in goat, rat or laying hen	No data available.	Metabolite found in plants at >10% TRR but the actual residues level is very low therefore not considered relevant for risk assessment. Toxicity would be covered by parent assessment as M13 data confirm this group of metabolites to be less toxic than parent.

Plant Metabolite	Maximum levels of residue in plants	Metabolite found in animal studies?	Mammalian toxicity data available?	Conclusion on relevance for avian risk assessment
Spiroxamine - hydroxy-N-oxide glucoside (M20) [Group A]	**Primary crops** Wheat Forage: 0.7% TRR; 0.08 mg/kg Straw: 2.0% TRR; 0.70 mg/kg Grain: not found Grapes Not found Banana Not found **Rotational crops** Swiss chard leaves (2.5% TRR; <0.01 mg/kg)) Wheat straw (2.1-2.6% TRR; 0.03 mg/kg) Turnip tops (8.4-10.4% TRR; 0.04 mg/kg)	Not found in goat, rat or laying hen	No data available.	Metabolite found in primary crops at <10% TRR with the exception of turnip tops but the actual residues level is very low. Therefore this metabolite is not considered relevant for risk assessment.
Spiroxamine - hydroxy-N-oxide malonyl glucoside (M21) [Group A]	**Primary crops** Wheat Forage: 2.0% TRR; 0.21 mg/kg Straw: 3.1% TRR; 2.57 mg/kg Grain: not found Grapes Not found Banana Not found **Rotational crops** Swiss chard leaves (1.6% TRR; <0.01 mg/kg) Wheat straw (4.4% TRR; 0.06 mg/kg) Turnip tops (1.7-3.7% TRR; <0.01 mg/kg)	Not found in goat, rat or laying hen	No data available.	Metabolite found in plants at <10% TRR therefore not considered relevant for risk assessment.

Plant Metabolite	Maximum levels of residue in plants	Metabolite found in animal studies?	Mammalian toxicity data available?	Conclusion on relevance for avian risk assessment
Spiroxamine-diol-diglycoside (M24) [Group B]	**Primary crops** Grapes (14.8% TRR – main component of metabolite group 12; 0.50 mg/kg) **Rotational crops** Swiss chard leaves (3.0% TRR; <0.01 mg/kg) Wheat straw (1.9-2.2% TRR; 0.020 mg/kg- Turnip roots (7.8% TRR; <0.01 mg/kg) Turnip tops (2.0-4.3% TRR; <0.01 mg/kg)	Not found in goat, rat or laying hen	No data available.	Metabolite found in grapes at >10% TRR but the actual residues level is very low therefore not considered relevant for risk assessment. Toxicity would be covered by parent assessment as M13 data confirm this group of metabolites to be less toxic than parent.
Spiroxamine - aminodiol (M28) [GROUP C]	**Primary crops** Wheat Not found Grapes 37.5% TRR; 4.91 mg/kg Banana Pulp: 31.2% TRR; 0.173 mg/kg Peel: 37.2% TRR; 2.45 mg/kg **Rotational crops** Leafy vegetables 3.9% TRR; 0.014 mg/kg Cereals 0.6% TRR; 0.024 mg/kg Root & tuber vegetables 4.9% TRR; 0.005 mg/kg	Found in rat at 2.2 – 5.7% of dose	Acute oral rat LD_{50} >550 <2000 mg/kg bw 28-day rat oral dietary NOAEL 28.4/31.4 mg/kg bw/day for males/females Developmental rat oral (gavage) NOAEL maternal toxicity 150 mg/kg bw/day and developmental NOAEL 30 mg/kg bw/day It was concluded that M28 is less toxic than the parent, spiroxamine in the rat with a *ca.* 15-fold, 9-fold and 2-fold increase in sub-acute, maternal and developmental NOAELs, respectively when compared to the spiroxamine equivalent studies.	Metabolite found in grapes and banana at >10% TRR therefore relevant for the risk assessment. Tox data are available and confirm that toxicity is less than parent. It is considered that this can also be extrapolated to birds therefore the avian reproductive risk assessment for spiroxamine covers the risk to this metabolite. M28 data used to represent the toxicity of all Group C metabolites.

Plant Metabolite	Maximum levels of residue in plants	Metabolite found in animal studies?	Mammalian toxicity data available?	Conclusion on relevance for avian risk assessment
Spiroxamine - aminodiol-N-oxide (M29) [GROUP C]	**Primary crops** Wheat Not found Grapes 0.1% TRR; 0.01 mg/kg Banana Not found **Rotational crops** Leafy vegetables 5.2% TRR; 0.021 mg/kg Root & tuber vegetables 4.8% TRR; 0.005 mg/kg	Not found in goat, rat or laying hen	No data available. Group C metabolites considered to be covered by available data for M28 which confirm that this metabolite is less toxic than spiroxamine.	Metabolite found in primary crops and rotational crops at <10% TRR therefore not considered relevant for risk assessment. Toxicity would be covered by parent assessment as M28 data confirm this group of metabolites to be less toxic than parent.
Spiroxamine - desethyl-aminodiol (M30) [GROUP C]	**Primary crops** Wheat Not found Grapes 1.1% TRR; 0.14 mg/kg Banana Pulp: 0.6% TRR; 0.003 mg/kg Peel: 0.9% TRR; 0.06 mg/kg	Not found in goat, rat or laying hen	No data available. Group C metabolites considered to be covered by available data for M28 which confirm that this metabolite is less toxic than spiroxamine.	Metabolite found in primary crops at <10% TRR therefore not considered relevant for risk assessment. Toxicity would be covered by parent assessment as M28 data confirm this group of metabolites to be less toxic than parent.
Spiroxamine - despropyl-aminodiol (M31) [GROUP C]	**Primary crops** Wheat Not found Grapes 1.2% TRR; 0.16 mg/kg Banana Pulp: 0.6% TRR; 0.003 mg/kg Peel: 0.9% TRR; 0.06 mg/kg **Rotational crops** Cereals 1.7% TRR; 0.034 mg/kg Root & tuber vegetables 6.1% TRR; 0.006 mg/kg	Not found in goat, rat or laying hen	No data available. Group C metabolites considered to be covered by available data for M28 which confirm that this metabolite is less toxic than spiroxamine.	Metabolite found in primary crops and rotational crops at <10% TRR therefore not considered relevant for risk assessment. Toxicity would be covered by parent assessment as M28 data confirm this group of metabolites to be less toxic than parent.

Plant Metabolite	Maximum levels of residue in plants	Metabolite found in animal studies?	Mammalian toxicity data available?	Conclusion on relevance for avian risk assessment
Spiroxamine-cyclohexanol-glucopyranosyl-pentose (M33) [GROUP B]	**Primary crops** Grapes (19.1% TRR; 0.650 mg/kg)	Not found in goat, rat or laying hen	No data available.	Metabolite found in plants at >10% TRR but the actual residues level is very low therefore not considered relevant for risk assessment. Toxicity would be covered by parent assessment as M13 data confirm this group of metabolites to be less toxic than parent.
Spiroxamine-cyclohexanol-glucopyranosyl-glucopyranosyl-pentose (M34) [GROUP B]	**Primary crops** Grapes (3.5% TRR; 0.130 mg/kg)	Not found in goat, rat or laying hen	No data available.	Metabolite found in plants at <10% TRR therefore not considered relevant for risk assessment.
Spiroxamine docosanoic acid ester (M35) [GROUP B]	**Primary crops** <u>Wheat</u> Not found <u>Grapes</u> 13.0% TRR; 0.44 mg/kg <u>Banana</u> Not found	Not found in goat, rat or laying hen	No data on M35. Group B metabolites considered to be covered by available data for M13 which confirm that this metabolite is less toxic than spiroxamine.	Metabolite found in grapes at >10% TRR. Available data with M13 used to cover this Group B metabolite and confirms that the toxicity is lower than that of the parent. Thus, the risk to M35 is considered to be covered by the assessment for parent.

Plant Metabolite	Maximum levels of residue in plants	Metabolite found in animal studies?	Mammalian toxicity data available?	Conclusion on relevance for avian risk assessment
Spiroxamine tetracosanoic acid ester (M36) [GROUP B]	**Primary crops** Wheat Not found Grapes 4.2% TRR; 0.14 mg/kg Banana Not found	Not found in goat, rat or laying hen	No data on M36. Group B metabolites considered to be covered by available data for M13 which confirm that this metabolite is less toxic than spiroxamine.	Metabolite found in primary crops at <10% TRR therefore not considered relevant for risk assessment. Toxicity would be covered by parent assessment as M13 data confirm this group of metabolites to be less toxic than parent.
Spiroxamine-cyclohexenol (M37) [GROUP B]	**Primary crops** Grapes (3.2% TRR; 0.11 mg/kg-hydrolysis product)	Not found in goat, rat or laying hen	No data available.	Metabolite found in plants at <10% TRR therefore not considered relevant for risk assessment.
Spiroxamine – N-formyl-despropyl (M38) [GROUP A]	**Primary crops** Not found **Rotational crops** Cereals 7.6% TRR; 0.243 mg/kg	Not found in goat, rat or laying hen	No data available.	Metabolite found in rotational crops only and at <10% TRR therefore not considered relevant for risk assessment.
Spiroxamine – hydroxy-despropyl glycoside (M39) [GROUP A]	**Rotational crops** Leafy vegetables 2.8% TRR; 0.019 mg/kg Cereals 5.9% TRR; 0.232 mg/kg Root & tuber vegetables 21.3% TRR; 0.063 mg/kg	Not found in goat, rat or laying hen	No data available.	Metabolite found in rotational crops at >10% TRR in root and tuber vegetables but the actual residues level is very low therefore not considered relevant for risk assessment.
Spiroxamine – hydroxy glycoside (M40) [GROUP A]	**Primary crops** Not found **Rotational crops** Leafy vegetables 4.7% TRR; 0.040 mg/kg Cereals 2.9% TRR; 0.088 mg/kg Root & tuber vegetables 7.6% TRR; 0.068 mg/kg	Not found in goat, rat or laying hen	No data available.	Metabolite found in rotational crops only and at <10% TRR therefore not considered relevant for risk assessment.

Plant Metabolite	Maximum levels of residue in plants	Metabolite found in animal studies?	Mammalian toxicity data available?	Conclusion on relevance for avian risk assessment
Spiroxamine – hydroxy-desethyl glycoside (M42) [GROUP A]	**Primary crops** Not found **Rotational crops** Leafy vegetables 1.6% TRR; 0.005 mg/kg Cereals 6.5% TRR; 0.129 mg/kg Root & tuber vegetables 14.6% TRR; 0.044 mg/kg	Not found in goat, rat or laying hen	No data available.	Metabolite found in rotational crops at >10% TRR in root and tuber vegetables but the actual residues level is very low therefore not considered relevant for risk assessment.
Spiroxamine – desethyl acid glycoside (M43) [GROUP A]	**Primary crops** Not found **Rotational crops** Leafy vegetables 1.8% TRR; 0.015 mg/kg Cereals 3.4% TRR; 0.088 mg/kg Root & tuber vegetables 5.7% TRR; 0.051 mg/kg	Not found in goat, rat or laying hen	No data available.	Metabolite found in rotational crops only and at <10% TRR therefore not considered relevant for risk assessment.
Spiroxamine – acid glycoside (M44) [GROUP A]	**Primary crops** Not found **Rotational crops** Leafy vegetables 4.6% TRR; 0.019 mg/kg Cereals 6.4% TRR; 0.126 mg/kg Root & tuber vegetables 11.6% TRR; 0.027 mg/kg	Not found in goat, rat or laying hen	No data available.	Metabolite found in rotational crops at >10% TRR in root and tuber vegetables but the actual residues level is very low therefore not considered relevant for risk assessment.
Spiroxamine – despropyl acid glycoside (M45) [GROUP A]	**Primary crops** Not found **Rotational crops** Leafy vegetables 5.5% TRR; 0.019 mg/kg Cereals 3.7% TRR; 0.145 mg/kg Root & tuber vegetables 9.1% TRR; 0.002 mg/kg	Not found in goat, rat or laying hen	No data available.	Metabolite found in rotational crops only and at <10% TRR therefore not considered relevant for risk assessment.

M01 and M02 were found in the rotational crop studies at >10% TRR (although at low absolute amounts) but these metabolites were also found in the hen metabolism study therefore the risk assessment of parent spiroxamine is considered to cover the risk to these metabolites.

M03 was found in wheat at >10% TRR. Toxicology data for M03 demonstrate that this metabolite is of similar or lower toxicity than spiroxamine. It is considered reasonable to assume that this would also be the case for birds therefore, assuming extrapolation from mammals to birds, the risk assessment of parent spiroxamine is considered to cover the risk to this metabolite.

M28 was found in grapes and banana at >10% TRR. Toxicology data are available for M28 and confirm that this metabolite is 15-fold, 9-fold and 2-fold less toxic in sub-acute, maternal and developmental parameters than spiroxamine. It is considered reasonable to assume that this would also be the case for birds therefore, assuming extrapolation from mammals to birds, it is considered that this metabolite will be less toxic to birds than spiroxamine therefore the risk from exposure to this metabolite is considered to be covered by the assessment for parent.

M35 was also found in grapes at >10%TRR. The toxicology data generated for M28 are considered to also cover this metabolite and confirm that the metabolite is less toxic than parent. Thus, the risk from exposure to this plant metabolite is considered to be covered by the assessment for parent.

M04, M05, M09, M14, M15, M16, M20, M21, M24, M29, M30, M31, M33, M34, M36, M37, M38, M39, M40, M42, M43, M44 and M45 were found in the crop metabolism studies at either <10% TRR or very low absolute amounts and were therefore not considered to be relevant for risk assessment. Specific dietary risk assessment for these plant metabolites of spiroxamine is therefore not considered to be necessary.

A detailed consideration of the metabolites of prothioconazole is not considered to be an integral part of the Renewal of Approval of spiroxamine. Thus, prothioconazole data have been used in the risk assessment of Prothioconazole + Spiroxamine EC 460 but the information has been taken from the 2007 EFSA Conclusion for prothioconazole. Prothioconazole-desthio is a metabolite of prothioconazole that occurred in amounts of >10% of the TRR on plant material and has been included in the residue definition. Therefore, a risk assessment is conducted. Furthermore, avian toxicity data are available. To facilitate the risk assessment for birds and mammals, a 100 % conversion of the parent compound prothioconazole into the metabolite prothioconazole-desthio has been assumed.

Dietary risk assessment for birds

Exposure

The proposed use of Prothioconazole + Spiroxamine EC 460 is for either one or two applications (14-day interval) to cereals (BBCH 30 - 69) at a maximum application rate of 1.25 L product/ha (equivalent to 375 g spiroxamine/ha and 200 g prothioconazole/ha). Risk assessments for both one and two applications at this rate have been conducted and are considered to cover all other proposed uses of this product.

Isomers

The risk assessments for birds & mammals involves potential chronic exposure of these organisms to residues in plants therefore it may be necessary to apply an uncertainty factor (UF) to the chronic avian and mammalian risk assessments. The acute risk assessment need not have an UF applied as exposure in this scenario is immediate. However, the chronic risk assessment considers exposure over a prolonged period therefore potential changes in isomeric ratio needs to be considered. For the bird and mammal risk assessments the same approach taken in the residues section for the consumer risk assessment, with respect to isomers, has been followed. Based on the current residues data set for spiroxamine, there are no indications of a significant change in isomer ratios therefore no additional factor need be applied to the risk assessments below (*i.e.* an UF of 1.0 has been used).

Risk assessment

The risk assessments have been conducted in accordance with the EFSA (2009) Bird & Mammal Guidance document. Each assessment starts with a screening step followed by a Tier I assessment if required. Finally, refined risk assessments have been presented where required.

The acute 'daily dietary dose' (DDD) is calculated by multiplying the shortcut value (SV) based on the 90th percentile residues by the application rate in kg a.s./ha.

DDD_A = application rate (kg a.s/ha) x SV_{90}

The long-term 'daily dietary dose' (DDD) is calculated by multiplying the SV based on the mean residues by the application rate in kg a.s./ha and (only for the long-term risk assessment) a time weighted average residue exposure (f_{twa}). The f_{twa} based upon a default DT_{50} of 10 days is 0.53, as given in EFSA guidance (2009).

DDD_{LT} = application rate (kg a.s./ha) x SV_m x f_{twa}

Acute risk is assessed by comparing the relevant daily dietary dose (DDD) values with the appropriate LD_{50} endpoint to give an acute Toxicity: Exposure Ratio (TER_A):

$$TER_A = \frac{LD_{50}\ (\text{mg/kg bw})}{\text{DDD}}$$

TER_A values which exceed a trigger value of 10 indicate an acceptable acute risk.

Derivation of the short-term toxicity exposure ratio is no longer a requirement according to the EFSA Guidance Document (2009) so a short-term risk assessment has not been presented. However, for spiroxamine the endpoint from the short-term dietary study with the bobwhite quail has been used in the acute risk assessment.

Long-term risk is assessed by comparing the long-term DDD values with the worst case NOAEL/NOEL from the reproduction studies, expressed as daily dietary dose, to give a long-term toxicity exposure ratio (TER_{LT}):

$$TER_{LT} = \frac{NOAEL\ (\text{mg/kg bw/day})}{\text{DDD (mg/kg bw/day)}}$$

TER_{LT} values which exceed a trigger value of 5 indicate acceptable chronic risk.

1 x 1.25 L/ha application of Prothioconazole & Spiroxamine EC 460

The screening step assessments for the acute and reproductive risks are presented below for spiroxamine, prothioconazole and prothioconazole-desthio.

Table CP 10.1.1-4 Screening step assessment for acute and long-term/reproductive risk to birds for the proposed use of Prothioconazole+Spiroxamine 460 in cereals – Spiroxamine

Intended use	Cereals				
Active substance/product	Spiroxamine / Prothioconazole + Spiroxamine 460				
Application rate (g a.s./ha)	1 × 375				
Acute toxicity (mg a.s./kg bw)	357				
TER criterion	10				
Crop scenario	Indicator species	SV_{90}	MAF_{90}	DDD_{90} (mg a.s./kg bw/d)	TER_A
Cereals	Small omnivorous bird	158.8	1.0	59.6	**>5.99**
Reprod. toxicity (mg a.s./kg bw/d)	5.40				
TER criterion	5				
Crop scenario	Indicator species	SV_m	MAF_m × TWA	DDD_m (mg a.s./kg bw/d)	TER_{LT}
Cereals	Small omnivorous bird	64.8	1.0 x 0.53	12.9	**0.419**

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity exposure ratio. TER values shown in bold fall below the relevant trigger

For the proposed use of Prothioconazole + Spiroxamine EC 460 on cereals (1 x 1.25 L product/ha at BBCH 30 - 69) potential acute and reproductive risks to birds from dietary exposure to spiroxamine have been identified (TER <10 and <5, respectively). A Tier I acute and reproductive risk assessment has therefore been conducted and presented below.

Table CP 10.1.1-5 Screening step assessment for acute and long-term/reproductive risk to birds for the proposed use of Prothioconazole+Spiroxamine 460 in cereals - Prothioconazole

Intended use	Cereals				
Active substance/product	Prothioconazole / Prothioconazole + Spiroxamine 460				
Application rate (g a.s./ha)	1 × 200				
Acute toxicity (mg a.s./kg bw)	>1413				
TER criterion	10				
Crop scenario	Indicator species	SV_{90}	MAF_{90}	DDD_{90} (mg a.s./kg bw/d)	TER_A
Cereals	Small omnivorous bird	158.8	1.0	31.8	>44.5
Reprod. toxicity (mg a.s./kg bw/d)	78				
TER criterion					
Crop scenario	Indicator species	SV_m	MAF_m × TWA	DDD_m (mg a.s./kg bw/d)	TER_{LT}
Cereals	Small omnivorous bird	64.8	1.0 x 0.53	6.87	11.4

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity exposure ratio

For the proposed use of Prothioconazole + Spiroxamine EC 460 on cereals (1 x 1.25 L product/ha at BBCH 30 - 69) the acute and reproductive risks to birds from dietary exposure to prothioconazole are considered to be acceptable (TER ≥10 and ≥5 for acute and reproductive risks, respectively). No further risk assessment for prothioconazole exposure is necessary.

Table CP 10.1.1-6 Screening step assessment for acute and long-term/reproductive risk to birds for the proposed use of Prothioconazole + Spiroxamine 460 in cereals – Prothioconazole-desthio

Intended use	Cereals				
Active substance/product	Prothioconazole-desthio / Prothioconazole + Spiroxamine 460				
Application rate (g a.s./ha)	1 × 200				
Acute toxicity (mg a.s./kg bw)	>297				
TER criterion	10				
Crop scenario	Indicator species	SV_{90}	MAF_{90}	DDD_{90} (mg a.s./kg bw/d)	TER_A
Cereals	Small omnivorous bird	158.8	1.0	31.8	**>9.35**
Reprod. toxicity (mg a.s./kg bw/d)	14.8				
TER criterion	5				
Crop scenario	Indicator species	SV_m	MAF_m / TWA	DDD_m (mg a.s./kg bw/d)	TER_{LT}
Cereals	Small omnivorous bird	64.8	1.0 x 0.53	6.87	**2.16**

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity exposure ratio. TER values shown in bold fall below the relevant trigger.

For the proposed use of Prothioconazole + Spiroxamine EC 460 on cereals (1 x 1.25 L product/ha at BBCH 30 - 69) potential acute and reproductive risks to birds from dietary exposure to prothioconazole-desthio have been identified (TER <10 and <5, respectively). A Tier I acute and reproductive risk assessment has therefore been conducted and presented below.

Acute risk assessment (Tier I)

Table CP 10.1.1-7 Tier I assessment for acute risk to birds for the proposed use of Prothioconazole + Spiroxamine 460 in cereals – Spiroxamine

Intended use	Cereals				
Active substance/product	Spiroxamine / Prothioconazole + Spiroxamine 460				
Application rate (g a.s./ha)	1 × 375				
Acute toxicity (mg a.s./kg bw)	>357				
TER criterion	10				
Crop scenario Growth stage	Generic focal species	SV_{90}	MAF_{90}	DDD_{90} (mg a.s./kg bw/d)	TER_A
Cereals BBCH 30-39	Small omnivorous bird "lark"	12.0	1.0	4.50	>79.3
Cereals BBCH >40	Small omnivorous bird "lark"	7.2	1.0	2.70	>132

SV: shortcut value; MAF: multiple application factor; DDD: daily dietary dose; TER: toxicity to exposure ratio.

For the proposed use of Prothioconazole + Spiroxamine EC 460 on cereals (1 x 1.25 L product/ha at BBCH 30 - 69) the acute risks to birds from dietary exposure to spiroxamine are considered to be acceptable (TER ≥10) for all relevant scenarios. No further acute risk assessment for spiroxamine is necessary for this use of Prothioconazole + Spiroxamine EC 460.

Table CP 10.1.1-8 Tier I assessment for acute risk to birds for the proposed use of Prothioconazole + Spiroxamine 460 in cereals – Prothioconazole-desthio

Intended use	Cereals				
Active substance/product	Prothioconazole-desthio / Prothioconazole + Spiroxamine 460				
Application rate (g a.s./ha)	1 × 200				
Acute toxicity (mg a.s./kg bw)	>297				
TER criterion	10				
Crop scenario Growth stage	Generic focal species	SV_{90}	MAF_{90}	DDD_{90} (mg a.s./kg bw/d)	TER_A
Cereals BBCH 30-39	Small omnivorous bird "lark"	12.0	1.0	2.40	>124
Cereals BBCH >40	Small omnivorous bird "lark"	7.2	1.0	1.44	>206

SV: shortcut value; MAF: multiple application factor; DDD: daily dietary dose; TER: toxicity to exposure ratio

For the proposed use of Prothioconazole + Spiroxamine EC 460 on cereals (1 x 1.25 L product/ha at BBCH 30 - 69) the acute risks to birds from dietary exposure to prothioconazole-desthio are considered to be acceptable (TER ≥10) for all relevant scenarios. No further acute risk assessment for prothioconazole-desthio is necessary for this use of Prothioconazole + Spiroxamine EC 460.

Reproductive risk assessment (Tier I)

Table CP 10.1.1-9 Tier I assessment for reproductive risk to birds for the proposed use of Prothioconazole + Spiroxamine 460 in cereals – Spiroxamine

Intended use	Cereals				
Active substance/product	Spiroxamine / Prothioconazole + Spiroxamine 460				
Application rate (g a.s./ha)	1 × 375				
Reprod. toxicity (mg a.s./kg bw/d)	5.40				
TER criterion	5				
Crop scenario Growth stage	Generic focal species	SV_m	MAF_m × TWA	DDD_m (mg a.s./kg bw/d)	TER_{LT}
Cereals BBCH 30-39	Small omnivorous bird "lark"	5.4	1.0 x 0.53	1.07	5.03
Cereals BBCH >40	Small omnivorous bird "lark"	3.3	1.0 x 0.53	0.656	8.23

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio

For the proposed use of Prothioconazole + Spiroxamine EC 460 on cereals (1 x 1.25 L product/ha at BBCH 30 - 69) the reproductive risks to birds from dietary exposure to spiroxamine are considered to be acceptable (TER ≥5) for all relevant scenarios. No further reproductive risk assessment for spiroxamine is necessary for this use of Prothioconazole + Spiroxamine EC 460.

Table CP 10.1.1-10 Tier I assessment for reproductive risk to birds for the proposed use of Prothioconazole+Spiroxamine 460 in cereals – Prothioconazole-desthio

Intended use	Cereals				
Active substance/product	Prothioconazole-desthio / Prothioconazole + Spiroxamine 460				
Application rate (g a.s./ha)	1 × 200				
Reprod. toxicity (mg a.s./kg bw/d)	14.8				
TER criterion	5				
Crop scenario Growth stage	Generic focal species	SV_m	MAF_m × TWA	DDD_m (mg a.s./kg bw/d)	TER_{LT}
Cereals BBCH 30-39	Small omnivorous bird "lark"	5.4	1.0 x 0.53	0.572	25.9
Cereals BBCH >40	Small omnivorous bird "lark"	3.3	1.0 x 0.53	0.350	42.3

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio.

For the proposed use of Prothioconazole + Spiroxamine EC 460 on cereals (1 x 1.25 L product/ha at BBCH 30 - 69) the reproductive risks to birds from dietary exposure to prothioconazole-desthio are considered to be acceptable (TER ≥ 5) for all relevant scenarios. No further reproductive risk assessment for prothioconazole-desthio is necessary for this use of Prothioconazole + Spiroxamine EC 460.

2 x 1.25 L/ha application of Prothioconazole & Spiroxamine EC 460

The screening step assessments for the acute and reproductive risks are presented below for spiroxamine, prothioconazole and prothioconazole-desthio.

Table CP 10.1.1-11 Screening step assessment for acute and long-term/reproductive risk to birds for the proposed use of Prothioconazole+Spiroxamine 460 in cereals - Spiroxamine

Intended use	Cereals				
Active substance/product	Spiroxamine / Prothioconazole + Spiroxamine 460				
Application rate (g a.s./ha)	2 × 375				
Acute toxicity (mg a.s./kg bw)	>357				
TER criterion	10				
Crop scenario	Indicator species	SV_{90}	MAF_{90}	DDD_{90} (mg a.s./kg bw/d)	TER_A
Cereals	Small omnivorous bird	158.8	1.2	71.5	**>5.00**
Reprod. toxicity (mg a.s./kg bw/d)	5.40				
TER criterion	5				
Crop scenario	Indicator species	SV_m	MAF_m × TWA	DDD_m (mg a.s./kg bw/d)	TER_{LT}
Cereals	Small omnivorous bird	64.8	1.4 x 0.53	18.0	**0.299**

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity exposure ratio. TER values shown in bold fall below the relevant trigger

For the proposed use of Prothioconazole + Spiroxamine EC 460 on cereals (2 x 1.25 L product/ha at BBCH 30 - 69) potential acute and reproductive risks to birds from dietary exposure to spiroxamine have been identified (TER <10 and <5, respectively). A Tier I acute and reproductive risk assessment has therefore been conducted and presented below.

Table CP 10.1.1-12 Screening step assessment for acute and long-term/reproductive risk to birds for the proposed use of Prothioconazole+Spiroxamine 460 in cereals - Prothioconazole

Intended use	Cereals				
Active substance/product	Prothioconazole / Prothioconazole + Spiroxamine 460				
Application rate (g a.s./ha)	2 × 200				
Acute toxicity (mg a.s./kg bw)	>1413				
TER criterion	10				
Crop scenario	Indicator species	SV_{90}	MAF_{90}	DDD_{90} (mg a.s./kg bw/d)	TER_A
Cereals	Small omnivorous bird	158.8	1.2	38.1	>32.1
Reprod. toxicity (mg/kg bw/d)	78				
TER criterion	5				
Crop scenario	Indicator species	SV_m	MAF_m × TWA	DDD_m (mg a.s./kg bw/d)	TER_{LT}
Cereals	Small omnivorous bird	64.8	1.4 x 0.53	9.62	8.11

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity exposure ratio.

For the proposed use of Prothioconazole + Spiroxamine EC 460 on cereals (2 x 1.25 L product/ha at BBCH 30 - 69) the acute and reproductive risks to birds from dietary exposure to prothioconazole are considered to be acceptable (TER ≥10 and ≥5 for acute and reproductive risks, respectively). No further risk assessment for prothioconazole exposure is necesary.

Table CP 10.1.1-13 Screening step assessment for acute and long-term/reproductive risk to birds for the proposed use of Prothioconazole + Spiroxamine 460 in cereals – Prothioconazole-desthio

Intended use	Cereals				
Active substance/product	Prothioconazole-desthio / Prothioconazole + Spiroxamine 460				
Application rate (g a.s./ha)	2 × 200				
Acute toxicity (mg a.s./kg bw)	>297				
TER criterion	10				
Crop scenario	Indicator species	SV_{90}	MAF_{90}	DDD_{90} (mg a.s./kg bw/d)	TER_A
Cereals	Small omnivorous bird	158.8	1.2	38.1	**>7.79**
Reprod. toxicity (mg a.s./kg bw/d)	14.8				
TER criterion	5				
Crop scenario	Indicator species	SV_m	MAF_m TWA	DDD_m (mg a.s./kg bw/d)	TER_{LT}
Cereals	Small omnivorous bird	64.8	1.4 x 0.53	9.62	**1.54**

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity exposure ratio. TER values shown in bold fall below the relevant trigger.

For the proposed use of Prothioconazole + Spiroxamine EC 460 on cereals (2 x 1.25 L product/ha at BBCH 30 - 69) potential acute and reproductive risks to birds from dietary exposure to prothioconazole-desthio have been identified. A Tier I acute and reproductive risk assessment has therefore been conducted and presented below.

Acute risk assessment (Tier I)

Table CP 10.1.1-14 Tier I assessment for acute risk to birds for the proposed use of Prothioconazole + Spiroxamine 460 in cereals – Spiroxamine

Intended use	Cereals				
Active substance/product	Spiroxamine / Prothioconazole + Spiroxamine 460				
Application rate (g a.s./ha)	2 × 375				
Acute toxicity (mg a.s./kg bw)	>357				
TER criterion	10				
Crop scenario Growth stage	Generic focal species	SV_{90}	MAF_{90}	DDD_{90} (mg a.s./kg bw/d)	TER_A
Cereals BBCH 30-39	Small omnivorous bird "lark"	12.0	1.2	5.40	>66.1
Cereals BBCH >40	Small omnivorous bird "lark"	7.2	1.2	3.24	>110

SV: shortcut value; MAF: multiple application factor; DDD: daily dietary dose; TER: toxicity to exposure ratio.

For the proposed use of Prothioconazole + Spiroxamine EC 460 on cereals (2 x 1.25 L product/ha at BBCH 30 - 69) the acute risks to birds from dietary exposure to spiroxamine are considered to be acceptable (TER ≥10) for all relevant scenarios. No further acute risk assessment for spiroxamine is necessary for this use of Prothioconazole + Spiroxamine EC 460.

Table CP 10.1.1-15 Tier I assessment for acute risk to birds for the proposed use of Prothioconazole + Spiroxamine 460 in cereals – Prothioconazole-desthio

Intended use	Cereals				
Active substance/product	Prothioconazole-desthio / Prothioconazole + Spiroxamine 460				
Application rate (g a.s./ha)	2 × 200				
Acute toxicity (mg a.s./kg bw)	>297				
TER criterion	10				
Crop scenario Growth stage	Generic focal species	SV_{90}	MAF_{90}	DDD_{90} (mg a.s./kg bw/d)	TER_A
Cereals BBCH 30-39	Small omnivorous bird "lark"	12.0	1.2	2.88	>103
Cereals BBCH >40	Small omnivorous bird "lark"	7.2	1.2	1.73	>172

SV: shortcut value; MAF: multiple application factor; DDD: daily dietary dose; TER: toxicity to exposure ratio

For the proposed use of Prothioconazole + Spiroxamine EC 460 on cereals (2 x 1.25 L product/ha at BBCH 30 - 69) the acute risks to birds from dietary exposure to prothioconazole-desthio are considered to be acceptable (TER ≥10) for all relevant scenarios. No further acute risk assessment for prothioconazole-desthio is necessary for this use of Prothioconazole + Spiroxamine EC 460.

Reproductive risk assessment (Tier I)

Table CP 10.1.1-16 Tier I assessment for reproductive risk to birds for the proposed use of Prothioconazole + Spiroxamine 460 in cereals – Spiroxamine

Intended use	Cereals				
Active substance/product	Spiroxamine / Prothioconazole + Spiroxamine 460				
Application rate (g a.s./ha)	2 × 375				
Reprod. toxicity (mg a.s./kg bw/d)	5.40				
TER criterion	5				
Crop scenario Growth stage	Generic focal species	SV_m	MAF_m × TWA	DDD_m (mg a.s./kg bw/d)	TER_{LT}
Cereals BBCH 30-39	Small omnivorous bird "lark"	5.4	1.4 x 0.53	1.50	**3.59**
Cereals BBCH >40	Small omnivorous bird "lark"	3.3	1.4 x 0.53	0.918	5.88

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio; TER values shown in bold fall below the relevant trigger

For the proposed use of Prothioconazole + Spiroxamine EC 460 on cereals (2 x 1.25 L product/ha at BBCH 30 - 69) the reproductive risks to birds from dietary exposure to spiroxamine were acceptable (TER ≥5) for the small omnivorous bird "lark" scenario at BBCH >40. However, potential reproductive risks have been identified for the small omnivorous bird "lark" scenario at BBCH 30 – 39 (TER <5). A refined risk assessment for this individual scenario has been presented below.

Table CP 10.1.1-17 Tier I assessment for reproductive risk to birds for the proposed use of Prothioconazole + Spiroxamine 460 in cereals – Prothioconazole-desthio

Intended use	Cereals
Active substance/product	Prothioconazole-desthio / Prothioconazole + Spiroxamine 460
Application rate (g a.s./ha)	2 × 200
Reprod. toxicity (mg a.s./kg bw/d)	14.8
TER criterion	5

Crop scenario Growth stage	Generic focal species	SV_m	$MAF_m \times TWA$	DDD_m (mg a.s./kg bw/d)	TER_{LT}
Cereals BBCH 30-39	Small omnivorous bird "lark"	5.4	1.4 x 0.53	0.801	18.5
Cereals BBCH >40	Small omnivorous bird "lark"	3.3	1.4 x 0.53	0.490	30.2

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio.

For the proposed use of Prothioconazole + Spiroxamine EC 460 on cereals (2 x 1.25 L product/ha at BBCH 30 - 69) the reproductive risks to birds from dietary exposure to prothioconazole-desthio are considered to be acceptable (TER ≥ 5) for all relevant scenarios. No further reproductive risk assessment for prothioconazole-desthio is necessary for this use of Prothioconazole + Spiroxamine EC 460.

Refined reproductive risk assessment for the small omnivorous bird "lark" scenario (BBCH 20 - 39) for exposure to spiroxamine following 2 x applications of Prothioconazole + Spiroxamine EC 460

For the refined risk assessment the skylark has been used as the focal species. In study M-290916-01-1 cereal fields in Germany, Poland, France and Italy were studied with regards to the composition of the bird communities present. The skylark was found to be the most abundant species present in cereal fields in Germany and Poland and in the top two most abundant species in France and Italy.

In study M-292641 cereal fields in Germany were studied with a particular emphasis on the yellowhammer, tree sparrow and quail. Although the skylark was not the focus of the study, the results of the observations demonstrated that the skylark was the most abundant bird species found within cereal fields.

In study M-297061-01-1 a radio-tracking program was carried out in a typical cereal cultivating region in Germany in spring to obtain ecological information on the skylark. The study again demonstrated that the skylark was the most abundant bird species found in cereals fields. 90th percentile PT values of 0.882 and 0.418 were determined for the skylark in winter cereals at BBCH 21-32 and freshly sown spring cereals, respectively.

In study M-291779-01-1 cereal fields in Southern Spain were also studied in order to define the bird community present. The skylark was found to be present in the monitored cereals field and, although not the most abundant species, the study confirms that skylarks do visit cereal fields in Spain.

Full details of all of these studies can be found in the study summaries below. The weight of evidence from all of these monitoring studies confirms that the skylark is a clear choice of focal species for the refined risk assessment for the use of Prothioconazole + Spiroxamine EC 460 in cereals.

In report M-557330-01-1, the radio-tracking data of 52 tracking sessions from 33 individual skylarks from three studies, including M-297061-01-1 discussed above, were pooled to estimate 21 day-PT values for the skylark in winter cereals in Spring. A 90th percentile PT value of 0.487 was determined

and this value has been applied to the refined risk assessment below in order replace the default assumption that birds obtain all of their food from within the treated area (*i.e.* PT of 1.0).

Several residues decline studies are available in which the residues of spiroxamine have been determined at frequent intervals following application of spiroxamine containing formulations to cereals. Report M-759383-01-1 summarises the kinetic analyses of all 24 available trials which have been conducted as part of five separate studies (M-301585-01-1, M-574326-01-1, M-578235-01-1, M-628347-02-1 and M-684671-01-1) covering wheat and barley plants in both Northern and Southern Europe. The individual validity of each study has been discussed in the evaluation section after each study summary but all of the trials used in this assessment were considered to be valid and suitable for use in the derivation of an overall crop dissipation half-life (DT_{50}) value. It was found that spiroxamine residues dissipated relatively quickly on cereals with an overall geomean DT_{50} of 3.03 days determined. A geomean DT_{50} value of 2.74 days was determined for Northern EU and a DT_{50} of 3.83 days for Southern EU. The overall geomean DT_{50} of 3.03 days has been applied to the refined risk assessment below and is considered to be suitably representative of the decline of spiroxamine residues throughout Europe. Note that the refined DT_{50} has only been applied to the crop leaves component of the diet as this is the matrix upon which the residues were determined. The DT_{50} of 3.03 days has been used in place of the default value of 10 days. As multiple applications have been considered, a combined MAF and f_{twa} value, using a moving time window approach, has been used to give a MAF x TWA of 0.377.

For the refined risk assessment the EFSA (2009) omnivorous diet for the lark has been considered of 25% crop leaves, 25% weed seeds and 50% ground invertebrates.

Table CP 10.1.1-18 Refinement of the small omnivorous bird scenario for the proposed use of Prothioconazole+Spiroxamine 460 in cereals – Spiroxamine - Skylark

Application rate	Food type	FIR/bw [a)]	RUD [b)]	MAF	f_{twa}	PT [d)]	Dep. factor [e)]	DDD [mg a.s./kg b.w./d]	Total DDD [f)]	TER [g)]
2 x 0.375 kg a.s./ha	Crop leaves	0.413	54.2	0.377 [c)]		0.487	0.5	0.211	0.634	8.52
	Weed seeds	0.113	40.2	1.4	0.53			0.308		
	Invertebrates (ground dwelling)	0.226	54.2	1.4	0.53			0.115		

a) Values calculated using EFSA (2009) dietary data
b) Default RUD values from EFSA (2009) Appendix F
c) A MAF×TWA value calculated using a moving time window and a DT_{50} value of 3.03 days
d) Refined 90th percentile PT value from focal species study
e) Deposition value based on 50% crop interception for cereals at principal growth stages ≥3 (Appendix E: EFSA, 2009)
f) Sum of DDD values for individual diet components
g) TER calculated based on reproductive endpoint of 5.40 mg a.s./kg bw/day

The reproductive risks to small omnivorous birds from dietary exposure to spiroxamine following the use of Prothioconazole + Spiroxamine Ec 460 on cereals at 2 x 1.25 L product/ha (BBCH 30 - 69) are considered to be acceptable (TER ≥5).

Combined toxicity

Mixture toxicity and exposure was calculated using the concentration addition model (CA model). For a mixture containing two active substances this can be expressed using the following equation:

$$LD_{50mix\text{-}CA} = 1 / (p^1/LD_{50}^1 + p^2/LD_{50}^2)$$

Where;
$LD_{50mix\text{-}CA}$: calculated mixture toxicity
[1] and [2] indicate active substance 1 and active substance 2, respectively
p: the proportion in the mix of each active as a fraction; Σp should always = 1

LD_{50}: experimentally determined EC_{50}/LC_{50}/NOEC

For the mixture toxicity risk assessment of Prothioconazole + Spiroxamine EC 460, it is the opinion of the notifier that only an acute risk assessment for combined exposure needs to be presented. Formulations break down into their respective components very shortly after they enter the environment therefore an assessment of the risk to any toxic effects of Prothioconazole + Spiroxamine EC 460 is only applicable to the acute exposure scenario in which the risks to birds and mammals over a very short time period are assessed. The chronic risk assessment considers the potential risks over much longer time periods, by which point the formulation will have broken down into the two individual active substances which then may behave differently. Thus, the mixed chronic risk of spiroxamine and prothioconazole applied as Prothioconazole + Spiroxamine EC 460 are considered to be covered by the risk assessments for the individual actives. However, an assessment of the contribution that each active substance makes to the overall chronic mixture toxicity has been presented below.

Careful selection of the toxicity endpoints is necessary for use in the predicted mixture toxicity calculations so that accurate predictions of toxicity can be made as well as meaningful comparisons with the measured formulation toxicity data. For spiroxamine, prothioconazole and prothioconazole-desthio the LD_{50} values of >357, >1413 and >297 mg a.s./kg bw, respectively have been used in the mixture toxicity calculations. All three of these endpoints have been taken from the short-term dietary toxicity studies and have therefore been conducted using the same test methods and, as such, the endpoints are considered suitably comparable for use in a mixture toxicity calculation. It is noted that all of these endpoints are unbound greater than ('>') values and therefore could lead to an inaccurate prediction of the mixture toxicity. However, as the endpoints are greater than values these would lead to a potential over-estimation of toxicity and therefore would provide a conservative estimate.

Formulation composition

Prothioconazole & Spiroxamine EC 460 has the following composition and relative proportions:

Spiroxamine: 300 g/L (65.2% or 0.652)

Prothioconazole: 160 g/L (34.8% or 0.348)

Predicted mixture toxicity

Using the acute LD_{50} endpoints for spiroxamine, prothioconazole and prothioconazole-desthio of >357, >1413 and >297 mg a.s./kg bw, respectively, the calculated mix-CA values in terms of total active substance have been determined and presented below. A predicted toxicity value for spiroxamine and prothioconazole has been calculated as well as a predicted value for spiroxamine and prothioconazole-desthio, assuming a worst case 100% conversion of prothioconazole to the more toxic metabolite.

Table CP 10.1.1-19 Calculated acute avian oral mixture toxicity in terms of total a.s. content for Prothioconazole + Spiroxamine EC 460

Combination	Endpoint	p^1/LD_{50}	p^2/LD_{50}	Calculated Mix-CA (mg total a.s./kg bw)[3]
Spiroxamine and prothioconazole	LD_{50}	0.00183	0.000246	482
Spiroxamine and prothioconazole-desthio	LD_{50}	0.00183	0.00117	334

[1] proportion of spiroxamine (p = 0.652)
[2] proportion of prothioconazole or prothioconazole-desthio (p = 0.348)
[3] $LD_{50mix\text{-}CA} = 1 / (p^1/LD_{50}{}^1 + p^2/LD_{50}{}^2)$
Calculated values have been rounded for presentation purposes

The predicted mixture toxicity of spiroxamine and prothioconazole in Prothioconazole & Spiroxamine EC 460 is 482 mg total a.s./kg bw. There is no experimentally determined acute oral toxicity value to compare the predicted toxicity value against. However, for the acute mammalian risk assessment for this formulation, the predicted acute oral mixture toxicity value was within a factor of 2 of the experimentally determined value therefore the principles of concentration addition are considered to be appropriate for this formulations and the predicted acute oral avian toxicity value is considered to be a reliable estimate.

The predicted mixture toxicity of spiroxamine and prothioconazole-desthio (assuming complete conversion of prothioconazole to the metabolite) in Prothioconazole & Spiroxamine EC 460 is 334 mg total a.s./kg bw.

Toxic units

The toxic unit (TU) of a mixture is defined as the sum of the TU of each individual substance in the mixture therefore the predicted data can also be examined for the contribution of the two separate active substances to the mixture toxic units.

If the toxicity of the mixture is largely explained by the toxicity of a single active substance, a sufficient protection level might be achieved by simply basing the risk assessment on the toxicity data for that single 'driver'. Hence, where CA provides a reliable estimate of the toxicity of the given mixture and the largest part of the sum of toxic units (*i.e.* ≥ 90 %) calculated for the measured mixture toxicity comes from a single active substance, it can be concluded that this component drives the overall mixture toxicity.

The toxic unit is calculated using the following equation:

$$\sum_{i=1}^{n} TU_i = \sum_{i=1}^{n} \frac{c_i}{ECx_i}$$

Where;
TU_i: Toxic units of component *i*
c_i: concentration of a mixture component *i*
ECx_i: concentration of component *i* provoking x% effect

The calculated toxic units for spiroxamine and prothioconazole along with the percentage contribution of each active to the overall toxicity of the mixture are presented below.

Table CP 10.1.1-20 Toxic unit calculations and contribution of each active substance to the predicted toxicity of Prothioconazole+Spiroxamine EC 460

Organism group	Active substance	C_i/ECx_i	TU_i	ΣTU_i	Contribution (%)
Acute toxicity	Spiroxamine	0.652 / 357	0.00183	0.00207	88.1
	Prothioconazole	0.348 / 1413	0.000246		11.9
Chronic toxicity	Spiroxamine	0.652 / 5.40	0.121	0.125	96.4
	Prothioconazole	0.348 / 78	0.00446		3.56

For acute toxicity, spiroxamine is the major contributor to the toxicity of the mixture of spiroxamine and prothioconazole but this is below the 90% threshold to be considered as the driver of the toxicity of the mixture. For chronic toxicity it is noted that spiroxamine is the clear driver of the mixture toxicity with 96.4% of the TU attributable to spiroxamine. Although a chronic mixture toxicity calculation has not been performed, based on the TU approach the chronic risk assessment for spiroxamine alone would cover the chronic risk assessment for the mixture.

Mixture toxicity - 1 x 1.25 L/ha application of Prothioconazole & Spiroxamine EC 460

Screening step

Table CP 10.1.1-21 Screening step assessment for acute risk to birds for the proposed use of Prothioconazole+Spiroxamine 460 in cereals – Combined mixture (SPX & PTZ)

Intended use	Cereals				
Active substance/product	Mixture SPX & PTZ / Prothioconazole + Spiroxamine 460				
Application rate (g total a.s./ha)	1 × 575 (375 SPX + 200 PTZ)				
Acute toxicity (mg total a.s./kg bw)	482				
TER criterion	10				
Crop scenario	Indicator species	SV_{90}	MAF_{90}	DDD_{90} (mg total a.s./kg bw/d)	TER_A
Cereals	Small omnivorous bird	158.8	1.0	91.3	**5.28**

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity exposure ratio. TER values shown in bold fall below the relevant trigger

Table CP 10.1.1-22 Screening step assessment for acute risk to birds for the proposed use of Prothioconazole+Spiroxamine 460 in cereals – Combined mixture (SPX & PTZ-desthio)

Intended use	Cereals				
Active substance/product	Mixture SPX & PTZ-desthio / Prothioconazole + Spiroxamine 460				
Application rate (g total a.s./ha)	1 × 575 (375 SPX + 200 PTZ)				
Acute toxicity (mg total a.s./kg bw)	334				
TER criterion	10				
Crop scenario Growth stage	Indicator species	SV_{90}	MAF_{90}	DDD_{90} (mg total a.s./kg bw/d)	TER_A
Cereals	Small omnivorous bird	158.8	1.0	91.3	**3.66**

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity exposure ratio. TER values shown in bold fall below the relevant trigger

Based on the screening step risk assessment for the combined effects of spiroxamine and prothioconazole as well as spiroxamine and prothioconazole-desthio, potential acute risks to birds have been identified following the proposed use of Prothioconazole + Spiroxamine EC 460 (1 x 1.25 L/ha) with TER values <10. A Tier I risk assessment has therefore been presented below.

Tier I Acute risk assessment

Table CP 10.1.1-23 Tier I assessment for acute risk to birds for the proposed use of Prothioconazole + Spiroxamine 460 in cereals – Combined toxicity (SPX & PTZ)

Intended use	Cereals				
Active substance/product	Mixture SPX & PTZ / Prothioconazole + Spiroxamine 460				
Application rate (g total a.s./ha)	1 × 575 (375 SPX + 200 PTZ)				
Acute toxicity (mg total a.s./kg bw)	482				
TER criterion	10				
Crop scenario Growth stage	Generic focal species	SV_{90}	MAF_{90}	DDD_{90} (mg total a.s./kg bw/d)	TER_A
Cereals BBCH 30-39	Small omnivorous bird "lark"	12.0	1.0	6.90	69.9
Cereals BBCH >40	Small omnivorous bird "lark"	7.2	1.0	4.14	116

SV: shortcut value; MAF: multiple application factor; DDD: daily dietary dose; TER: toxicity to exposure ratio

Table CP 10.1.1-24 Tier I assessment for acute risk to birds for the proposed use of Prothioconazole + Spiroxamine 460 in cereals – Combined toxicity (SPX & PTZ-desthio)

Intended use	Cereals				
Active substance/product	Mixture SPX & PTZ-desthio / Prothioconazole + Spiroxamine 460				
Application rate (g total a.s./ha)	1 × 575 (375 SPX + 200 PTZ)				
Acute toxicity (mg total a.s./kg bw)	334				
TER criterion	10				
Crop scenario Growth stage	Generic focal species	SV_{90}	MAF_{90}	DDD_{90} (mg total a.s./kg bw/d)	TER_A
Cereals BBCH 30-39	Small omnivorous bird "lark"	12.0	1.0	6.90	48.4
Cereals BBCH >40	Small omnivorous bird "lark"	7.2	1.0	4.14	80.7

SV: shortcut value; MAF: multiple application factor; DDD: daily dietary dose; TER: toxicity to exposure ratio

Based on the Tier I acute risk assessment for the combined effects of spiroxamine and prothioconazole as well as spiroxamine and prothioconazole-desthio, the acute risks to birds have been demonstrated to be acceptable (TER ≥10) following the proposed use of Prothioconazole + Spiroxamine EC 460 (1 x 1.25 L/ha). No further acute risk assessment for combined effects is considered to be necessary.

Mixture toxicity - 2 x 1.25 L/ha application of Prothioconazole & Spiroxamine EC 460

Screening step

Table CP 10.1.1-25 Screening step assessment for acute risk to birds for the proposed use of Prothioconazole+Spiroxamine 460 in cereals – Combined mixture (SPX & PTZ)

Intended use	Cereals				
Active substance/product	Mixture SPX & PTZ / Prothioconazole + Spiroxamine 460				
Application rate (g total a.s./ha)	2 × 575 (375 SPX + 200 PTZ)				
Acute toxicity (mg total a.s./kg bw)	482				
TER criterion	10				
Crop scenario	Indicator species	SV_{90}	MAF_{90}	DDD_{90} (mg total a.s./kg bw/d)	TER_A
Cereals	Small omnivorous bird	158.8	1.2	110	**4.40**

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity exposure ratio. TER values shown in bold fall below the relevant trigger

Table CP 10.1.1-26 Screening step assessment for acute risk to birds for the proposed use of Prothioconazole+Spiroxamine 460 in cereals – Combined mixture (SPX & PTZ-desthio)

Intended use	Cereals				
Active substance/product	Mixture SPX & PTZ-desthio / Prothioconazole + Spiroxamine 460				
Application rate (g total a.s./ha)	2 × 575 (375 SPX + 200 PTZ)				
Acute toxicity (mg total a.s./kg bw)	334				
TER criterion	10				
Crop scenario	Indicator species	SV_{90}	MAF_{90}	DDD_{90} (mg total a.s./kg bw/d)	TER_A
Cereals	Small omnivorous bird	158.8	1.2	110	**3.05**

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity exposure ratio. TER values shown in bold fall below the relevant trigger

Based on the screening step risk assessment for the combined effects of spiroxamine and prothioconazole as well as spiroxamine and prothioconazole-desthio, potential acute risks to birds have been identified following the proposed use of Prothioconazole + Spiroxamine EC 460 (2 x 1.25 L/ha) with TER values <10. A Tier 1 risk assessment has therefore been presented below.

Tier 1 Acute risk assessment

Table CP 10.1.1-27 Tier I assessment for acute risk to birds for the proposed use of Prothioconazole + Spiroxamine 460 in cereals – Combined toxicity (SPX & PTZ)

Intended use	Cereals				
Active substance/product	Mixture SPX & PTZ / Prothioconazole + Spiroxamine 460				
Application rate (g total a.s./ha)	2 × 575 (375 SPX + 200 PTZ)				
Acute toxicity (mg total a.s./kg bw)	482 mg				
TER criterion	10				
Crop scenario Growth stage	Generic focal species	SV_{90}	MAF_{90}	DDD_{90} (mg total a.s./kg bw/d)	TER_A
Cereals BBCH 30-39	Small omnivorous bird "lark"	12.0	1.2	8.28	58.2
Cereals BBCH >40	Small omnivorous bird "lark"	7.2	1.2	4.97	97.0

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio.

Table CP 10.1.1-28 Tier I assessment for acute risk to birds for the proposed use of Prothioconazole + Spiroxamine 460 in cereals – Combined toxicity (SPX & PTZ-desthio)

Intended use	Cereals				
Active substance/product	Mixture SPX & PTZ-desthio / Prothioconazole + Spiroxamine 460				
Application rate (g total a.s./ha)	2 × 575 (375 SPX + 200 PTZ)				
Acute toxicity (mg total a.s./kg bw)	334				
TER criterion	10				
Crop scenario Growth stage	Generic focal species	SV_{90}	MAF_{90}	DDD_{90} (mg total a.s./kg bw/d)	TER_A
Cereals BBCH 30-39	Small omnivorous bird "lark"	12.0	1.2	8.28	40.3
Cereals BBCH >40	Small omnivorous bird "lark"	7.2	1.2	4.97	67.2

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio.

Based on the Tier I acute risk assessment for the combined effects of spiroxamine and prothioconazole as well as spiroxamine and prothioconazole-desthio, the acute risks to birds have been demonstrated to be acceptable (TER ≥ 10) following the proposed use of Prothioconazole + Spiroxamine EC 460 (2 x 1.25 L/ha). No further acute risk assessment for combined effects is considered to be necessary.

Risks for birds through drinking water

In addition to dietary items, birds may also be exposed to residues occurring in drinking water. The daily dietary dose (DDD) values used in the standard dietary risk assessments do not encompass drinking water and therefore the potential risk from this exposure route has not been covered in the dietary risk assessment. Two scenarios are considered:

Leaf scenario

This scenario assumes pooling of spray solution in leaf whorls following application and is relevant only for certain crops and growth stages *e.g.* leafy vegetables forming heads or with a morphology that might facilitate collection of spray and from BBCH principle growth stage 4 until harvest. This scenario is not considered to be applicable to the proposed use on cereals.

Puddle scenario

This scenario considers puddles occurring on the soil surface following a rainfall event after application and is considered possible in all crop types.

In accordance with the EFSA Guidance Document (2009), based on the characteristics of the exposure scenario and standard assumptions for water uptake by animals no specific requirement for drinking water exposure calculation and TER determination based on the puddle scenario is required:

- for substances with a Koc <500 L/kg (less sorptive), if the ratio of application rate (g a.s./ha) to effective endpoint mg a.s./kg bw/d does not exceed 50;
- for substances with a Koc ≥ 500 L/kg (more sorptive), if the ratio of application rate (g a.s./ha) to effective endpoint mg a.s./kg bw/d does not exceed 3000.

The geomean Koc for spiroxamine is 4111 L/kg. For prothioconazole and prothioconazole-desthio, mean Koc values of 1765 L/kg and 575 L/kg, respectively have been used (EFSA Scientific Report (2007) 106, 1-98). Thus, spiroxamine, prothioconazole and prothioconazole-desthio all belong to the group of more sorptive substances.

For spiroxamine the ratio calculations are based on two applications of 375 g a.s./ha. For prothioconazole and prothioconazole-desthio the ratio calculations are based on two applications of 200 g a.s./ha

Table CP 10.1.1-29 Ratios of effective application rate (AR_{eff}) to acute and long-term endpoints for spiroxamine, prothioconazole and prothioconazole-desthio following the proposed use of Prothioconazole & Spiroxamine EC 460- puddle scenario

Test substance	AR_{eff} (g/ha)[a]	Toxicological endpoint (mg a.s./kg bw/d)	Ratio (AR_{eff}/endpoint)	Trigger
Acute				
Spiroxamine	450	LD_{50} >337	1.26	3000
Prothioconazole	240	LD_{50} >1413	0.170	
Prothioconazole-desthio	240	LD_{50} >297	0.808	
Long-term				
Spiroxamine	525	NOAEL 5.40	97.2	3000
Prothioconazole	280	NOEL 78	3.59	
Prothioconazole-desthio	280	NOEL 14.8	18.9	

[a] AR_{eff} = based on an application rate of 375 or 200 g a.s./ha for spiroxamine or prothioconazole / prothioconazole-desthio, respectively with a MAF of 1.2 and 1.4 applied for acute and reproductive risk assessments, respectively

The ratios for both acute and reproductive risks are below the relevant trigger of 3000 for spiroxamine, prothioconazole and prothioconazole-desthio therefore a quantitative risk assessment is not necessary. Thus, there are no unacceptable risks to birds from exposure to either spiroxamine, prothioconazole or prothioconazole-desthio *via* drinking water.

Secondary poisoning

The Log P_{ow} of spiroxamine is 2.79 and 2.98 at pH 7 for diastomers A and B, respectively but at pH 9 these value are 4.88 and 5.08, respectively. Thus the trigger value of 3 for a secondary poisoning risk assessment is met.

The Log Pow of prothioconazole was determined to be 3.82 at pH 7. Thus a risk assessment for a generic earthworm eating bird and a generic fish eating bird has been performed to evaluate the risk of secondary poisoning.

Consideration of secondary poisoning risk due to metabolites

The Log P_{ow} of spiroxamine-desethyl (M01) is 2.41, 1.97 and >3.64 at pH 4, 7 and 9, respectively. The Log P_{ow} of spiroxamine-despropyl (M02) is 1.95, 1.41 and >3.44 at pH 4, 7 and 9, respectively. The Log P_{ow} of spiroxamine-N-oxide (M03) is 2.82, 2.59 and 2.63 at pH 4, 7 and 9, respectively. The Log P_{ow} of spiroxamine-carboxylic acid (M06) is 0.45, -0.25 and 0.10 at pH 4, 7 and 9, respectively. Thus, an assessment of the potential risk from bioconcentration of spiroxamine-desethyl (M01) and spiroxamine-despropyl (M02) also needs to be addressed in the risk assessment.

Prothioconazole-desthio and prothioconazole-S-methyl are the major soil and major aquatic metabolites of prothioconazole. Both metabolites have Log Pow values >3, i.e. 3.04 and 4.30, respectively. Therefore the risk of secondary poisoning for earthworm eating and for fish eating birds has been considered for both metabolites. Another major aquatic metabolite of prothioconazole, 1,2,4-triazole, has a log Pow of <3, therefore no risk to birds due to bioaccumulation has to be expected from this metabolite.

Risk assessment for earthworm-eating birds *via* secondary poisoning

According to EFSA (2009), the risk for vermivorous birds is assessed for a bird of 100 g body weight with a daily food consumption of 104.6 g. Bioaccumulation in earthworms is estimated based on predicted concentrations in soil.

To achieve a concise risk assessment, the risk envelope approach is applied whereby the maximum application rate of 2 x 1.25 L/ha has been considered. For spiroxamine, M01 and M02, the $PEC_{soil\ accumulation}$ or the 21-day TWA PEC_{soil} value, whichever is highest, has been used in the risk assessment. There are no avian reproductive toxicity data available for M01 and M02 therefore the NOAEL of 5.40 mg/kg bw/day for spiroxamine has been used as a surrogate value.

For prothioconazole, prothioconazole-desthio and prothioconazole-S-methyl, the 21-day TWA PEC_{soil} values have been taken from the spiroxamine RAR (Spiroxamine dRAR, Volume 3, Annex B.9) along with the Log P_{ow} values. K_{oc} values and the toxicity endpoints have been taken from the EFSA Conclusion for prothioconazole (EFSA Scientific Report (2007) 106, 1 - 98). For Prothioconazole-S-methyl there are no avian reproductive toxicity data therefore the prothioconazole endpoint has been used as a surrogate.

The secondary poisoning risk assessments for earthworm-eating birds from exposure to spiroxamine, KWG 4168-desethyl (M01), KWG 4168-despropyl (M02), prothioconazole, prothioconazole-desthio and prothioconazole-S-methyl are presented in the tables below.

Table CP 10.1.1-30 Assessment of the risk for earthworm-eating birds due to exposure to spiroxamine *via* bioaccumulation in earthworms (secondary poisoning)

Parameter	Spiroxamine	Comments
PEC_{soil} (mg a.s./kg soil)	0.157	21-day TWA PEC_{soil}
Log P_{ow} / P_{ow}	4.0 / 10000	Mean value of 4.0 has been used based on the values for diastomers A and B at pH 7 and 9
K_{oc}	4111	Mean
f_{oc}	0.02	Default

Parameter	Spiroxamine	Comments
BCF_{worm}	1.47	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw})$ $= (0.84 + 0.012 \times P_{ow}) / f_{oc} \times K_{oc}$
PEC_{worm}	0.231	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg a.s./kg bw/d)	0.242	$DDD = PEC_{worm} \times 1.05$
NOEL (mg a.s./kg bw/d)	5.40	From study M-007470-03-1
TER_{LT}	22.3	Acceptable risks (TER>5)

Table CP 10.1.1-31 Assessment of the risk for earthworm-eating birds due to exposure to spiroxamine-desethyl (M01) via bioaccumulation in earthworms (secondary poisoning)

Parameter	Spiroxamine-desethyl	Comments
PEC_{soil} (mg/kg soil)	0.030	Accumulation PEC_{soil} used as worst-case
Log P_{ow} / P_{ow}	3.64 / 4365	
K_{oc}	3271	Mean
f_{oc}	0.02	Default
BCF_{worm}	0.814	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw})$ $= (0.84 + 0.012 \times P_{ow}) / f_{oc} \times K_{oc}$
PEC_{worm}	0.0244	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	0.0256	$DDD = PEC_{worm} \times 1.05$
NOEL (mg/kg bw/d)	5.40	Value determined for spiroxamine used as a surrogate
TER_{LT}	211	Acceptable risks (TER>5)

Table CP 10.1.1-32 Assessment of the risk for earthworm-eating birds due to exposure to spiroxamine-despropyl (M02) *via* bioaccumulation in earthworms (secondary poisoning)

Parameter	Spiroxamine-despropyl	Comments
PEC_{soil} (mg/kg soil)	0.021	Accumulation PEC_{soil} used as worst-case
Log P_{ow} / P_{ow}	3.44 / 2754	-
K_{oc}	2695	Mean
f_{oc}	0.02	Default
BCF_{worm}	0.629	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw})$ $= (0.84 + 0.012 \times P_{ow}) / f_{oc} \times K_{oc}$
PEC_{worm}	0.0132	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	0.0139	$DDD = PEC_{worm} \times 1.05$
NOEL (mg/kg bw/d)	5.40	Value determined for spiroxamine used as a surrogate

TER_{LT}	389	Acceptable risks (TER>5)

Table CP 10.1.1-33 Assessment of the risk for earthworm-eating birds due to exposure to prothioconazole *via* bioaccumulation in earthworms (secondary poisoning)

Parameter	Prothioconazole	Comments
PEC_{soil} (mg a.s./kg soil)	0.028	21-day TWA PEC_{soil} from spiroxamine RAR
Log P_{ow} / P_{ow}	3.82 / 6607	Spiroxamine RAR
K_{oc}	1765	Mean value taken from EFSA Scientific Report (2007) 106, 1-98
f_{oc}	0.02	Default
BCF_{worm}	2.27	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw})$ $= (0.84 + 0.012 \times P_{ow}) / f_{oc} \times K_{oc}$
PEC_{worm}	0.0636	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg a.s./kg bw/d)	0.0667	$DDD = PEC_{worm} \times 1.05$
NOEL (mg a.s./kg bw/d)	78	EFSA Scientific Report (2007) 106, 1-98
TER_{LT}	1169	Acceptable risks (TER>5)

Table CP 10.1.1-34 Assessment of the risk for earthworm-eating birds due to exposure to prothioconazole-desthio *via* bioaccumulation in earthworms (secondary poisoning)

Parameter	Prothioconazole-desthio	Comments
PEC_{soil} (mg/kg soil)	0.066	21-day TWA PEC_{soil} from spiroxamine RAR
Log P_{ow} / P_{ow}	3.04 / 1096	Spiroxamine RAR
K_{oc}	575	Mean value taken from EFSA Scientific Report (2007) 106, 1-98
f_{oc}	0.02	Default
BCF_{worm}	1.22	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw})$ $= (0.84 + 0.012 \times P_{ow}) / f_{oc} \times K_{oc}$
PEC_{worm}	0.0803	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	0.0844	$DDD = PEC_{worm} \times 1.05$
NOEL (mg/kg bw/d)	14.8	EFSA Scientific Report (2007) 106, 1-98
TER_{LT}	175	Acceptable risks (TER>5)

Table CP 10.1.1-35 Assessment of the risk for earthworm-eating birds due to exposure to prothioconazole-S-methyl *via* bioaccumulation in earthworms (secondary poisoning)

Parameter	Prothioconazole-S-methyl	Comments
PEC_{soil} (mg/kg soil)	0.018	21-day TWA PEC_{soil} from spiroxamine RAR
Log P_{ow} / P_{ow}	4.30 / 19953	Spiroxamine RAR
K_{oc}	2556	Mean value taken from EFSA Scientific Report (2007) 106, 1-98.
f_{oc}	0.02	Default
BCF_{worm}	4.70	$BCF_{worm/soil} = (PEC_{worm,ww} / PEC_{soil,dw}) = (0.84 + 0.012 \times P_{ow}) / f_{oc} \times K_{oc}$
PEC_{worm}	0.0846	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	0.0888	$DDD = PEC_{worm} \times 1.05$
NOEL (mg/kg bw/d)	78	Value determined for prothioconazole used as a surrogate
TER_{LT}	878	Acceptable risks (TER>5)

For the secondary poisoning risk assessments for earthworm-eating birds from exposure to spiroxamine, M01, M02, prothioconazole, prothioconazole-desthio and prothioconazole-S-methyl the TER values are >5 thereby demonstrating an acceptable risk to birds *via* this route of exposure.

Risk assessment for fish-eating birds *via* secondary poisoning

According to EFSA (2009), the risk for piscivorous birds is assessed for a bird of 1000 g body weight with a daily food consumption of 159 g. Bioaccumulation in fish is estimated based on predicted concentrations in surface water.

To achieve a concise risk assessment, the risk envelope approach is applied. The highest Step 3 TWA PEC_{sw} of 2.029 µg a.s./L for spiroxamine has been used in the risk assessment. For M01 the highest Step 2 PEC_{sw} value of 0.826 µg/L has been used and for M02 the highest Step 2 PEC_{sw} value of 0.699 µg/L has been used. For M01 and M02 there are no BCF values available therefore the BCF value determined for spiroxamine (87 L/kg) has been used. Furthermore, there are no avian reproductive toxicity data available for M01 and M02 therefore the NOAEL of 5.40 mg/kg bw/day for spiroxamine has been used as a surrogate value.

For prothioconazole the highest Step 2 PEC_{sw} value of 1.263 µg a.s./L has been used. For prothioconazole-desthio the highest Step 3 max PEC_{sw} value of 1.244 µg/L has been used and for prothioconazole-S-methyl the highest Step 2 TWA PEC_{sw} value of 0.542 µg/L has been used. These values have been taken from the current draft RAR for Spiroxamine (Spiroxamine dRAR, Volume 3, Annex B.9). The fish BCF values for prothioconazole and prothioconazole-desthio of 19.7 and 65 L/kg, respectively have been used with NOEL values of 78 and 14.8 mg/kg bw/day, respectively. For prothioconazole-S-methyl there are no BCF or avian reproductive toxicity data available therefore the values for prothioconazole have been used as a surrogate.

The secondary poisoning risk assessments for fish-eating birds from exposure to spiroxamine, KWG 4168-desethyl (M01), KWG 4168-despropyl (M02), prothioconazole, prothioconazole-desthio and prothioconazole-S-methyl are presented in the tables below.

Table CP 10.1.1-36 Assessment of the risk for fish-eating birds due to exposure to spiroxamine *via* bioaccumulation in fish (secondary poisoning)

Parameter	Spiroxamine	Comments
PEC_{sw} (mg a.s./L)	0.002029	FOCUS Step 3 TWA PEC_{sw} (calculated for Spring cereals: D1 ditch, 2 x 375 g a.s./ha, early application)
PEC_{water} (mg a.s./L)	0.002029	TWA PEC_{sw} value used
BCF_{fish}	87	From study M-006479-01-1
PEC_{fish}	0.177	$PEC_{fish} = PEC_{water} \times BCF_{fish}$
Daily dietary dose (mg a.s./kg bw/d)	0.0281	$DDD = PEC_{fish} \times 0.159$
NOAEL (mg a.s./kg bw/d)	5.40	From study M-007470-03-1
TER_{LT}	192	Acceptable risks (TER>5)

Table CP 10.1.1-37 Assessment of the risk for fish-eating birds due to exposure to spiroxamine-desethyl (M01) *via* bioaccumulation in fish (secondary poisoning)

Parameter	Spiroxamine-desethyl	Comments
PEC_{sw} (mg/L)	0.000826	FOCUS Step 2 maximum PEC_{sw} (calculated for Spring/Winter cereals; 2 x 375 g a.s./ha)
PEC_{water} (mg/L)	0.000438	$PEC_{water} = \max PEC_{sw} \times f_{twa}$, where $f_{twa} = 0.53$; in line with approach in EFSA (2009)
BCF_{fish}	87	Value determined for spiroxamine used as a surrogate
PEC_{fish}	0.0381	$PEC_{fish} = PEC_{water} \times BCF_{fish}$
Daily dietary dose (mg/kg bw/d)	0.00606	$DDD = PEC_{fish} \times 0.159$
NOAEL (mg/kg bw/d)	5.40	Value determined for spiroxamine used as a surrogate
TER_{LT}	892	Acceptable risks (TER>5)

Table CP 10.1.1-38 Assessment of the risk for fish-eating birds due to exposure to spiroxamine-despropyl (M02) *via* bioaccumulation in fish (secondary poisoning)

Parameter	Spiroxamine-despropyl	Comments
PEC_{sw} (mg/L)	0.000699	FOCUS Step 2 maximum PEC_{sw} (calculated for Spring/Winter cereals; 2 x 375 g a.s./ha)
PEC_{water} (mg/L)	0.000370	$PEC_{water} = \max PEC_{sw} \times f_{twa}$, where $f_{twa} = 0.53$; in line with approach in EFSA (2009)
BCF_{fish}	87	Value determined for spiroxamine used as a surrogate
PEC_{fish}	0.0322	$PEC_{fish} = PEC_{water} \times BCF_{fish}$
Daily dietary dose (mg/kg bw/d)	0.00512	$DDD = PEC_{fish} \times 0.159$

NOAEL (mg/kg bw/d)	5.40	Value determined for spiroxamine used as a surrogate
TER_{LT}	1054	Acceptable risks (TER>5)

Table CP 10.1.1-39 Assessment of the risk for fish-eating birds due to exposure to prothioconazole *via* bioaccumulation in fish (secondary poisoning)

Parameter	Prothioconazole	Comments
PEC_{sw} (mg a.s./L)	0.001263	FOCUS Step 2 TWA PEC_{sw} (calculated for cereals)
PEC_{water} (mg a.s./L)	0.001263	TWA PEC_{sw} value used
BCF_{fish}	19.7	EFSA Scientific Report (2007) 106, 1-98
PEC_{fish}	0.0249	$PEC_{fish} = PEC_{water} \times BCF_{fish}$
Daily dietary dose (mg a.s./kg bw/d)	0.00396	$DDD = PEC_{fish} \times 0.159$
NOEL (mg a.s./kg bw/d)	78	EFSA Scientific Report (2007) 106, 1-98
TER_{LT}	19716	Acceptable risks (TER>5)

Table CP 10.1.1-40 Assessment of the risk for fish-eating birds due to exposure to Prothioconazole-desthio *via* bioaccumulation in fish (secondary poisoning)

Parameter	Prothioconazole-desthio	Comments
PEC_{sw} (mg/L)	0.001244	FOCUS Step 3 maximum PEC_{sw} (Winter cereals, R4 Stream)
PEC_{water} (mg/L)	0.000659	$PEC_{water} = \max PEC_{sw} \times f_{twa}$, where $f_{twa} = 0.53$; in line with approach in EFSA (2009)
BCF_{fish}	65	EFSA Scientific Report (2007) 106, 1-98
PEC_{fish}	0.0429	$PEC_{fish} = PEC_{water} \times BCF_{fish}$
Daily dietary dose (mg/kg bw/d)	0.00681	$DDD = PEC_{fish} \times 0.159$
NOEL (mg/kg bw/d)	14.8	EFSA Scientific Report (2007) 106, 1-98
TER_{LT}	2172	Acceptable risks (TER>5)

Table CP 10.1.1-41 Assessment of the risk for fish-eating birds due to exposure to Prothioconazole-S-methyl *via* bioaccumulation in fish (secondary poisoning)

Parameter	Prothioconazole-S-methyl	Comments
PEC_{sw} (mg/L)	0.000542	FOCUS Step 2 TWA PEC_{sw} (calculated for cereals)
PEC_{water} (mg/L)	0.000542	TWA PEC_{sw} value used
BCF_{fish}	19.7	Value determined for prothioconazole used as a surrogate

Parameter	Prothioconazole-S-methyl	Comments
PEC_{fish}	0.0107	$PEC_{fish} = PEC_{water} \times BCF_{fish}$
Daily dietary dose (mg/kg bw/d)	0.00170	$DDD = PEC_{fish} \times 0.159$
NOEL (mg/kg bw/d)	78	Value determined for prothioconazole used as a surrogate
TER_{LT}	45944	Acceptable risks (TER>5)

For the secondary poisoning risk assessments for fish-eating birds from exposure to spiroxamine, M01, M02, prothioconazole, prothioconazole-desthio and prothioconazole-S-methyl the TER values are >5 thereby demonstrating an acceptable risk to birds *via* this route of exposure.

Biodiversity

No relevant scientifically peer-reviewed open literature could be found on spiroxamine or its major metabolites, from an ecotoxicological perspective, on birds. Therefore, it is considered that the potential impact of the active substance on biodiversity and the ecosystem, including potential indirect effects *via* alteration of the food web, are covered by the risk assessment for birds in this section and in the ED hazard assessment.

CP 10.1.1.1 Acute oral toxicity

No avian acute oral toxicity data are available for Prothioconazole + Spiroxamine EC 460. However, the available data on the individual active substances spiroxamine and prothioconazole are considered to be sufficient to assess the risk of this formulation to birds.

CP 10.1.1.2 Higher tier data on birds

Residues studies

The following residues decline data are available and considered relevant to the proposed use of Prothioconazole + Spiroxamine EC 460 in cereals.

Data Point:	KCP 10.1.1.2/29
Report Author:	[redacted]
Report Year:	2020
Report Title:	Spiroxamine: Kinetic assessment of residue decline study
Report No:	047836-KIN1
Document No:	M-759383-01-1
Guideline(s) followed in study:	FOCUS (2014) and EFSA (2019)
Deviations from current test guideline:	None
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	No, not conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The decline of spiroxamine residues in wheat and barley plants has been investigated in the field in five studies conducted at trial locations in northern and southern Europe. The objective of this project was to describe the calculation of kinetic endpoints from these studies according to the guidance of FOCUS

(2014). Modelling DT_{50} values were calculated for use in deriving a crop dissipation half-life endpoint. Calculation of persistence endpoints was not considered necessary as there are no relevant study triggers for comparison.

The spiroxamine modelling DT_{50} values ranged from 1.14 to 6.93 days. The overall geometric mean was 3.03 days, with geomean for northern EU of 2.74 days and southern EU of 3.83 days.

I. Materials and Methods

Study Design

The decline of spiroxamine residues in wheat and barley plants has been investigated in the field in five studies conducted at trial locations in northern and southern Europe (M-301585-01-1, M-574326-01-1, M-578235-01-1, M-628347-02-1 and M-684671-01-1). The objective of this project was to describe the calculation of kinetic endpoints from these studies according to the guidance of FOCUS (2014). Modelling DT_{50} values were calculated for use in deriving a crop dissipation half-life endpoint. Calculation of persistence endpoints was not considered necessary as there are no relevant study triggers for comparison.

Input data were generated according to the data handling recommendations of FOCUS (2014). The kinetic modelling of the laboratory data was conducted using the CAKE (version 3.4) software package.

The FOCUS (2014) flowchart for calculating modelling endpoints has been followed. The residue decline behaviour of spiroxamine has been investigated in the field in twenty European trial sites. The results of this study have been used to determine the half-life in the crop canopy for spiroxamine under field conditions.

Modelling endpoints representing the decline rates of spiroxamine in wheat and barley plants have been calculated in accordance with the guidance of FOCUS (2014) and EFSA (2019), and are summarised in the tables below:

II. Results and Discussion

Table CP 10.1.1.2/29-1 Overall summary of modelling endpoints for spiroxamine

Study	Trial	DT_{50} (days)	$\chi 2$ err %	Kinetic model
M-301585-01-1	UK R2007 0671/5 first application	3.02	1.09	SFO
	UK R2007 0671/5 second application	2.5	9.08	FOMC
	Geomean UK R2007 0671/5 value	2.75	-	-
	Sweden R2007 0698/7*	3.28	6.69	FOMC
	Southern France R-2007 0699/5 first application*	1.7	3.06	SFO
	Southern France R 2007 0699/5 second application*	1.76	3.73	FOMC
	Geomean Southern France R 2007 0699/5 value*	1.73	-	-
	Italy R 2007/2	2.57	0.725	SFO

Study	Trial	DT_{50} (days)	$\chi 2$ err %	Kinetic model
	first application			
	Italy R 2007/2 second application	2.56	10.2	SFO
	Geomean Italy R 2007/2 value	2.56	-	-
M-574326-01-1	France 16-2958-01*	1.14	5.67	FOMC
	Germany 16-2958-02	2.91	6.53	SFO
	The Netherlands 16-2958-03*	3.34	10.4	FOMC
	Germany 16-2958-04*	6.3	2.03	DFOP
M-578235-01-1	France 16-2952-01*	3.3	3.47	FOMC
	Spain 16-2952-02	4.93	4.27	DFOP
	Italy 16-2952-04	6.93	3.91	DFOP
	Portugal 16-2952-04*	6.34	6.8	HS
M-628347-02-1	Germany 17-2950-01*	6.23	4.52	DFOP
	Northern France 17-2950-02*	1.27	6.49	FOMC
	The Netherlands 17-2950-03	3.81	2.01	HS
	Belgium 17-2950-04	1.73	8.92	SFO
M-684671-01-1	Germany E19RP088-01	4.3	9.32	SFO
	Germany E19RP088-02	3	12.5	SFO
	Belgium E19RP088-03	2.38	7.04	SFO
	The Netherlands E19RP088-04	1.32	11.6	FOMC
Average (all data)	3.48			
Geometric mean (all data)	3.03			
Average (excluding trials with >1mm rainfall within 24 hours of application)	3.33			
Geometric mean (excluding trials with >1mm rainfall within 24 hours of application	3.01			

[1] For these trials, two applications were applied

*Trials with rainfall within 24 hours of application

Table CP 10.1.1.2/29-2 Overall summary of modelling endpoints for spiroxamine on Northern EU sites

Trial	DT_{50} (days)	χ2 err %	Kinetic model
UK R2007 0671/5 first application[1]	3.02	1.09	SFO
UK R2007 0671/5 second application[1)]	2.5	9.08	FOMC
Geomean UK R2007 0671/5 value	2.75		
Sweden R2007 0698/7	3.28	6.69	FOMC
France 16-2958-01	1.14	5.67	FOMC
Germany 16-2958-02	2.99	6.53	SFO
The Netherlands 16-2958-03	3.34	10.4	FOMC
Germany 16-2958-04	6.3	2.03	DFOP
Germany 17-2950-01	6.23	4.57	DFOP
Northern France 17-2950-02	1.27	6.49	FOMC
The Netherlands 17-2950-03	3.84	2.01	HS
Belgium 17-2950-04	4.73	8.92	SFO
Germany E19RP088-01	4.3	9.32	SFO
Germany E19RP088-02	3	12.5	SFO
Belgium E19RP088-03	2.38	7.04	SFO
The Netherlands E19RP088-04	1.32	11.6	FOMC
Average	3.13		
Geomean	2.74		

[1] For these trials, two applications were applied

Table CP 10.1.1.2/29-3 Overall summary of modelling endpoints for spiroxamine on Southern EU sites

Trial	DT_{50} (days)	χ2 err %	Kinetic model
Southern France R 2007 0699/5 first application[1]	1.7	3.06	SFO
Southern France R 2007 0699/5 second application[1]	1.76	3.73	FOMC

Geomean Southern France R 2007 0699/5 value*	1.73	-	-
Italy R 2007/2 first application[1]	2.57	0.725	FOMC
Italy R 2007/2 second application[1]	2.56	10.2	SFO
Geomean Italy R 2007/2 value	2.56	-	-
France 16-2952-01	3.3	3.47	FOMC
Spain 16-2952-02	4.93	4.27	DFOP
Italy 16-2952-04	6.93	3.91	DFOP
Portugal16-2952-04	6.34	6.8	HS
Average	4.30		
Geomean	3.83		

[1] For these trials, two applications were applied

III. Conclusion

The residue decline behaviour of spiroxamine in wheat and barley plants has been investigated in the field in twenty European trial sites.

The spiroxamine modelling DT_{50} values ranged from 1.14 to 6.93 days. The overall geometric mean was 3.03 days, with geomean for northern EU of 2.74 days and southern EU of 3.83 days.

Assessment and conclusion by applicant:

This report has been generated in order to compile the decline data of spiroxamine on cereals from a total of 24 residues decline trials conducted as part of five studies. Residues of spiroxamine have been determined at frequent intervals following application of spiroxamine containing formulations to cereals. Studies M-301585-01-1, M-574326-01-1, M-578235-01-1, M-628347-02-1 and M-684671-01-1, covering wheat and barley plants in both Northern and Southern Europe, have been summarised and assessed separately below. The individual validity of each study has been discussed in the evaluation section after each study summary but all of the trials used in this assessment were considered to be valid and suitable for use in the derivation of an overall crop dissipation half-life (DT_{50}) value. It was found that spiroxamine residues dissipated relatively quickly on cereals with an overall geomean DT_{50} of 3.03 days determined. A geomean DT_{50} value of 2.74 days was determined for Northern EU and a DT_{50} of 3.83 days for Southern EU. These determined DT_{50} values have been used as part of the refined risk assessments to replace the default DT_{50} of 10 days with a more realistic experimentally determined value. It is noted that only the parts of the focal species diet that relates to cereals has used these refined values with the other parts of the diets still using the default value.

The report has followed the guidance set out in the FOCUS (2014) *Generic guidance for estimating persistence and degradation kinetics from environmental fate studies on pesticides in EU registration, Version 1.1*. The analysis has also used up to date modelling software (CAKE). Thus, the results are considered to be valid and reliable and therefore suitable for use in risk assessment in situations where its use has been sufficiently justified.

Data Point:	KCP 10.1.1.2/01
Report Author:	[redacted]; [redacted]
Report Year:	2008
Report Title:	Determination of the residues of KWG 4168 in/on spring barley after spraying of KWG 4168 (500 EC) in the field in United Kingdom, Sweden, Southern France, and Italy
Report No:	RA-2648/07
Document No:	M-301585-01-1
Guideline(s) followed in study:	91/414/EEC of July 15, 1991, 7029/VI/95 rev. 5 (1997-07-22)
Deviations from current test guideline:	No deviations (from study plan) occurred which had a negative influence on the study results
Previous evaluation:	yes, evaluated and accepted RAR (2010) Submitted and evaluated as part of the report M-301999-01-1
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The purpose of the presented study was to determine the magnitude of residues of KWG 4168 in/on spring barley green material sampled at various intervals after two spray applications with KWG 4168 (500 EC) at approx. 0.75 L/ha each, early in the growing season. The first application was made approx. at growth stage BBCH 25 (5 tillers visible), and the second approx. 14 days later. Two trials each were conducted in northern and southern Europe, with the individual trials located in the United Kingdom, Sweden, southern France, and Italy.

This study comprises four supervised field residue trials with spring barley in the United Kingdom, Sweden, Southern France, and Italy. A treated and an untreated plot were used for each trial.

The study was conducted according to the relevant guidelines. No residues were found in the control samples. The results for the recovery samples were in the range of 90 - 100%, in conformance with guidelines.

I. Materials

Test Material Spiroxamine EC 500E G

Lot/Batch #: PF90087683

Purity: 500 g/L (nominal), 501 g/L (analysed)

Description: Not stated

Stability of test compound: Not stated

Reanalysis/Expiry date: 31 January 2010

Density: Not stated

Treatments

Test rates: Two applications of 0.75 L/ha

Solvent/vehicle: Water was used as a carrier (200 L/ha)

Analysis of test concentrations:	Determination of spiroxamine was conducted using high performance liquid chromatography with mass spectrophotometric detection (HPLC-MS/MS).

Test design

Test area:	Four residue trials in the UK (sandy loam), Sweden, Southern France (clay silt) and Italy (silty sand). Each trial consisted of a treated and untreated plot. Plots ranged from 100 to 360 m^2
Sampling:	The sample material to be analysed was green material. Samples were collected on -14, -9, -4, -0, 0, 1, 2, 3, 5, 7, 10 and 14 after last treatment (DALT) from the UK and Italy study sites, on Day -14, -9, -4, -0, 0, 1, 2, 3, 8, 7, 10 and 14 DALT from the Sweden study site and -14, -9, -4, -0, 0, 2, 2, 3, 5, 7, 10 and 14 DALT from the Southern France site.
Duration of test:	14 days

Study design

The purpose of the presented study was to determine the magnitude of residues of KWG 4168 in/on spring barley green material sampled at various intervals after two spray applications with KWG 4168 (500 EC) at approx. 0.75 L/ha each, early in the growing season. The first application was made approx. at growth stage BBCH 25 (5 tillers visible), and the second approx. 14 days later. Two trials each were conducted in northern and southern Europe, with the individual trials located in the United Kingdom, Sweden, southern France, and Italy.

The test site for the field phase R 2007 0671/5 was Bayer CropScience Ltd., 230 Cambridge Science Park, Milton Road, Cambridge, CB4 0 WB. The test site for the field phase R 2007 0698/7 was Bayer Sverige AB S-20211 Malmö. The test site for the field phase R 2007 0699/5 was Bayer CropScience France 16 rue Jean Marie Leclerc F-69337 Lyon cedex 09, CP 310. The test site for the field part R 2007 0700/2 was Bayer CropScience Italy I-20156 Milano.

Samples were taken, prepared in the field where necessary, transported and stored according to EC guidance 7029/VI/95 rev.5 (1997-07-22). The field sub-samples from all trials were stored deep-frozen within 24 hours after sampling and until dispatch to the Laboratory for Sampling, Preparation Technique and Sample Logistics (PVTL), Bayer CropScience AG in D-40789 Monheim am Rhein. All field sub-samples were shipped by deep-freeze lorry and arrived at PVTL in good condition. The field sub-samples were stored in a freezer at -18°C or below until preparation of the examination samples. For the preparation of examination samples, the deep-frozen field sub-samples were shredded with dry ice in a cutter. Representative parts of the shredded samples were transferred into polystyrene boxes separately for analysis (UP samples) and archiving (UR samples) and stored at -18°C or below until analysis.

The analytical method was developed for the determination of residues of BYF00587, Prothioconazole, and the metabolites BYF00587-desmethyl and JAU6476-desthio (SXX0665) in/on plant materials. 5 g of sample was extracted with a mixture of acetonitrile/water (4/1; v/v, containing cysteine hydrochloride) using a blender. After filtration of the extract, the stable isotopically labelled analytes were added. The solution was made up to volume, diluted and subjected to reversed phase HPLC-MS/MS without a further clean-up step. In modification M001 to method 01013, spiroxamine (KWG 4168) was extracted from barley (grain, green material, straw) as described above and detected in ESI positive mode. Residues were quantified using internal stable labelled standards. The LOQ for all compounds, defined as the lowest validated fortification level, was 0.01 mg/kg for all sample materials.

Analytical method

Samples of green material were analysed using the validated analytical method M-301585-01-1, report reference M-301585-01-1 **(see Doc MCP Section 5)**.

II. Results and Discussion

Temperature for Trial R 2007 0671/5 (UK) ranged between 9 – 14 °C and daily rainfall ranged between 0 – 26 mm. Temperature for Trial R 2007 0698/7 (Sweden) ranged between 10 – 21 °C and daily rainfall ranged between 0 – 4 mm. Temperature for Trial R 2007 0699/5 (France) ranged between 12 – 19 °C and daily rainfall ranged between 0 – 15 mm. Temperature for Trial R 2007 0700/2 (Italy) ranged between 15 – 23 °C and daily rainfall ranged between 0 – 6 mm.

The study was conducted according to the relevant guidelines. No residues were found in the control samples. The results for the recovery samples were in the range of 70-100%, in conformance with guidelines.

The analytical method 01013 was developed for the determination of residues of BYF00587, Prothioconazole, and the metabolites BYF00587-desmethyl and JAU6476-desthio (SXX0665) in/on plant materials. 5 g of sample was extracted with a mixture of acetonitrile/water (4/1; v/v, containing cysteine hydrochloride) using a blender. After filtration of the extract, the stable isotopically labeled analytes were added. The solution was made up to volume, diluted and subjected to reversed phase HPLC-MS/MS without a further clean-up step.

In modification M001 to method 01013, spiroxamine (KWG 4168) was extracted from barley (grain, green material, straw) as described above and detected in ESI positive mode. Residues were quantified using internal stable labelled standards.

The LOQ for all compounds, defined as the lowest validated fortification level, was 0.01 mg/kg for all sample materials.

Table CP 10.1.1.2/01-1 Analytical results of control samples for KWG 4168, test system: spring barley

Trial No.	Growth stage (BBCH)	DALT	Sample material	KWG 4168 (mg/kg)
United Kingdom R 2007 0671/5	25	-14	Green material	< 0.01
	31	-0	Green material	< 0.01
	43	14	Green material	< 0.01
Sweden R 2007 0698/7	24	-14	Green material	< 0.01
	37	-0	Green material	< 0.01
	61	14	Green material	< 0.01
Southern France R 2007 0699/5	25	-14	Green material	< 0.01
	31	-0	Green material	< 0.01
	51	14	Green material	< 0.01
Italy R 2007 0700/2	25	-14	Green material	< 0.01
	32	-0	Green material	< 0.01
	59	14	Green material	< 0.01

DALT = Days after last application

Table CP 10.1.1.2/01-2 Analytical results of treated samples for KWG 4168, test system: spring barley

Trial No.	Growth stage (BBCH)	DALT	Sample material	KWG 4168 (mg/kg)
United Kingdom R 2007 0671/5	25	-14	Green material	18
	30	-9	Green material	5.6
	30	-4	Green material	1.9
	31	-0	Green material	0.85
	31	0	Green material	13
	31	1	Green material	4.3
	31	2	Green material	3.5
	32	3	Green material	2.7
	32	5	Green material	2.4
	32	7	Green material	1.4
	33	10	Green material	0.76
	42	14	Green material	0.34
Sweden R 2007 0698/7	24	-14	Green material	14
	24	-9	Green material	2.4
	31	-4	Green material	0.41
	37	0	Green material	14
	37	1	Green material	7.3
	37	2	Green material	6.0
	37	3	Green material	4.7
	39	5	Green material	2.9
	43	8	Green material	1.8
	49	10	Green material	1.3
	61	14	Green material	0.68
Southern France R 2007 0699/5	25	-14	Green material	12
	29	-9	Green material	1.5
	30	-4	Green material	0.43
	31	-0	Green material	0.16
	31	0	Green material	8.8
	31	2	Green material	2.2
	31	2	Green material	2.0
	31	3	Green material	1.4
	31	5	Green material	1.1
	31	7	Green material	0.85
	32	10	Green material	0.59

Trial No.	Growth stage (BBCH)	DALT	Sample material	KWG 4168 (mg/kg)
	51	15	Green material	0.25
Italy R 2007 0700/2	25	-14	Green material	19
	25	-9	Green material	5.0
	30	-4	Green material	1.2
	32	-0	Green material	0.39
	32	0	Green material	11
	32	1	Green material	6.9
	32	2	Green material	5.3
	33	3	Green material	4.7
	37	5	Green material	3.0
	39	7	Green material	1.9
	54	10	Green material	0.98
	59	14	Green material	0.68

DALT = days after last treatment

III. Conclusion

The study was conducted according to the relevant guidelines. No residues were found in the control samples. The results for the recovery samples were in the range of 70 - 100%, in conformance with guidelines.

Assessment and conclusion by applicant:

There is no formal test guideline for the conduct of residues decline trials but some guidance is provided in the EFSA Guidance document on Risk Assessment for Birds and Mammals (2009) as well as the more recent EFSA Technical Report on general recurring issues in ecotoxicology (EFSA supporting publication 2019:EN-1673). Up to date regulatory intelligence on the expectations of the test design of such trials has also been taken in to consideration.

The study comprised four trials over two countries in NEU (Sweden and the UK) and two countries in SEU (Sothern France and Italy). The crop used was spring barley which is highly relevant to the proposed representative GAP for cereals. The application rate was high enough in order to achieve sufficiently high residues of spiroxamine at the start of the trials, thereby allowing for a good decline curve to derive DT_{50} values from.

The analytical sampling regime adopted in the trials is considered to be suitable to derive reliable DT_{50} values, with sampling timepoints typically conducted before application and on Days 0, 1, 2, 3, 5, 7, 10 and 14. This regime is considered to meet the expectations for a robust residues decline trial, even with the relatively short expected DT_{50} for spiroxamine.

Weather data were adequately recorded from the nearest weather station.

Overall, the data are considered reliable and suitable for inclusion in the derivation of refined DT_{50} values in cereals for use in Bird & Mammal risk assessment.

Report M-759383-01-1 presents the results of the kinetic modeling for the spiroxamine data measured for these trials as well as the other available decline studies on cereals. An overall DT_{50} value for

spiroxamine has been determined using all of these data. As part of the analysis, trials that measured >1 mm rainfall in the first 24 hours were both included and excluded in order to assess the impact that this rainfall may have had on the overall DT_{50} value. It was concluded that there was very little variation in the mean DT_{50} value when these trials were either included or excluded. Thus, it is considered that any rainfall recorded in these trials has not adversely affected the results achieved therefore these trials are considered to be valid and can be included in the determination of the residue decline for spiroxamine.

Data Point:	KCP 10.1.1.2/02
Report Author:	[redacted]
Report Year:	2008
Report Title:	Half-life of spiroxamine residues on crops (barley)
Report No:	M-301999-01-1
Document No:	M-301999-01-1
Guideline(s) followed in study:	not specified
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and accepted RAR (2010)
GLP/Officially recognised testing facilities:	not applicable
Acceptability/Reliability:	Yes

Executive Summary

The objective of this document was to present the residue values from residues trials in Europe on barley and then the calculation of the DT_{50}.

In study RA-2648/07, four field trials were carried out in the 2007 season, two in the northern zone of Europe (Great Britain and Sweden) and two in the southern zone (Italy and France), to determine the residues of spiroxamine in barley at various stages of early crop growth. Two applications of Spiroxamine EC 500 were made, the first at growth stage BBCH 24-25 and the second 14 days later, at BBCH 31-37. Samples were taken at multiple intervals between days 0 and 14 after the final application (as well as 4 intervals following the first application) in order to determine the DT_{50} values for parent spiroxamine.

The calculation of the DT_{50} was based on the analysis results of the residue studies performed in Europe on barley. In four trials, half-life values after two applications ranged between 3.12 and 3.51 days. No regional differences were evident in the degradation times. The mean residue DT_{50} in barley plants after two applications of spiroxamine (KWG 4168) is 3.4 days. Additional calculations after a single application, based on fewer measured data points, yielded a similar result, with DT_{50} values ranging from 1.96-3.16 days.

I. Methods

Study Design

Samples from barley plants were taken at different times after the last treatment, generally at 0, 1, 2, 3, 5, 7, 10, and 14 days. The sample material was the whole plant without roots ("green material") in all cases. In addition, samples were also taken approx. 0, 5, 10, and 14 days after the first application.

A first-order single-exponential dissipation of spiroxamine residues was assumed, which can be described by equation 1:

$$c(t) = c_0 \exp(-k\,t)$$

where c(t) is the residue at time t, c0 is the initial residue at day 0, and k is the dissipation rate (d-1). This equation was fitted to the experimental data by changing k systematically to minimise the sum of squared differences between measured and calculated values. The curve fitting was conducted by means of the Solver non-linear optimisation tool supplied with Microsoft® Excel. The dissipation half-life time (DT_{50}) can be calculated from the dissipation rate k by (equation 2):

$$DT_{50} = (\ln 2)/k$$

II. Results and Discussion

In each of the trials, there are 8 data points for green material sampled on days 0-14 after the final treatment with residue values >LOQ (0.01 mg/kg), thus allowing the calculation of the DT_{50} for parent spiroxamine in all cases. The calculation was conducted using a single exponential first-order model.

The fit of the single-exponential dissipation curve was good for all field trials, as indicated by the correlation coefficients (r^2), which were >0.90 in all cases except one (southern France, trial R 2007 0699/5, r^2=0.87).

Although there were fewer data points following the first application, the half-life of the spiroxamine residues was also calculated for this scenario, as visual evaluation of the decline following the first applications (samples taken from "day -14" to "day -0") showed great similarity to the pattern seen after the second application. The fit of the single exponential model was good in all cases (r^2 >0.95).

The calculated DT_{50} values for spiroxamine in barley are presented in the table below.

Table CP 10.1.1.2/02-1 Half-lives for spiroxamine residues on barley plants (green material)

Region	Country *Trial No.*	DT_{50} (d) after application no.:	
		1	2
Northern EU	Great Britain *R 2007 0671/5*	3.16	3.12
	Sweden *R 2007 0698/7*	1.96	3.47
Southern EU	France *R 2007 0699/5*	2.28	3.36*
	Italy *R 2007 0700/2*	2.49	3.51

*The correlation coefficient (r^2) was 0.87 in this trial.

Table CP 10.1.1.2/02-2 Application and residue summary for spiroxamine (KWG 4168) in/on spring barley in Europe H

Country Trial No.*	Formulation type	No.	Application		Portion analysed	DALT	Residues of KWG 4168 [mg/kg]
			Kg a.s./ha	Kg a.s./hL			
Trials in northern Europe							
United Kingdom *R 2007 0671/5*	EC 500	2	0.375	0.1875	Green material	-14	18
					Green material	-9	5.6
					Green material	-4	1.9
					Green material	0	0.83
					Green material	0	3
					Green material	1	4.3
					Green material	2	3.5
					Green material	3	2.7
					Green material	5	2.4
					Green material	7	1.4
					Green material	10	0.76
					Green material	14	0.34
Sweden *R 2007 0698/7*	EC 500	2	0.375	0.125	Green material	-14	14
					Green material	-9	2.4
					Green material	-4	0.41
					Green material	0	14
					Green material	1	7.3
					Green material	2	6.0
					Green material	3	4.7
					Green material	5	2.9
					Green material	8	1.8
					Green material	10	1.3
					Green material	14	0.68

Country Trial No.*	Formulation type	No.	Application		Portion analysed	DALT	Residues of KWG 4168 [mg/kg]
			Kg a.s./ha	Kg a.s./hL			
Trials in northern Europe							
Trials in southern Europe							
France *R 2007 0699/5*	EC 500	2	0.375	0.125	Green material	-14	12
					Green material	-9	1.5
					Green material	-4	0.43
					Green material	-0	0.16
					Green material	0	8.8
					Green material	2	2.2
					Green material	2	2.0
					Green material	3	1.4
					Green material	5	1.1
					Green material	8	0.85
					Green material	10	0.59
					Green material	14	0.25
Italy *R 2007 0700/2*	EC 500	2	0.375	0.125	Green material	-14	19
					Green material	-9	5.0
					Green material	-4	1.2
					Green material	0	0.39
					Green material	0	11
					Green material	1	6.9
					Green material	2	5.3
					Green material	3	4.7
					Green material	5	3.0
					Green material	7	1.9
					Green material	10	0.98
					Green material	14	0.68

DALT = days after last treatment

III. Conclusion

The calculation of the DT_{50} was based on the analysis results of the residue studies performed in Europe on barley. In four trials, half-life values after two applications ranged between 3.12 and 3.51 days. No regional differences were evident in the degradation times.

The mean residue DT_{50} in barley plants after two applications of spiroxamine (KWG 4168) is 3.4 days.

Additional calculations after a single application, based on fewer measured data points, yielded a similar result, with DT_{50} values ranging from 1.96-3.16 days.

Assessment and conclusion by applicant:

This study was an internal document produced in order to determine DT_{50} values from the residues trials conducted in Europe on barley as part of study M-301585-01-1.

The determined DT_{50} values are considered to be valid but have not been used in the risk assessment for Prothioconazole & Spiroxamine EC 460. Instead, new kinetics modelling has been conducted for the data generated in study M-301585-01-1 as well as several other residues studies. The results of this modelling has been presented in report M-759383-01-1.

This study is considered to be supporting information only.

Data Point:	KCP 10.1.1.2/30
Report Author:	[redacted]
Report Year:	2015
Report Title:	Final report - Residue decline of spiroxamine and prothioconazole on arthropods after spray application on cereal fields in western Germany
Report No:	P13046
Document No:	M-529934-01-1
Guideline(s) followed in study:	Regulation (EC) No 1107/2009; EFSA Guidance Document on Risk Assessment for Birds and Mammals (2009)
Deviations from current test guideline:	None
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The residue decline of spiroxamine, prothioconazle and the metabolite JAU 6476-desthio in foliage dwelling and flying arthropods was determined after application with the formulated product Input Classic® (containing 300g/L spiroxamine and 160 g/L prothioconazole) at the application rate of 1 × 1.25 L product/ha on cereal fields in Western Germany.

Three cereal fields (winter wheat) were selected, representative of fields in the region in terms of size and structure, with potentially high populations of arthropods. One replicate with a size of approximately 1 ha was established in each field.

The residue decline of spiroxamine on leaf-dwelling arthropods and flying insects declined with initial fluctuations. Measured residue concentrations of prothioconazolwe rapidly declined after application in foliage-dwelling arthropods and flying insects. The metabolite JAU 6476-desthio reached its maximum concentrations on DAT+2 on foliage-dwellers before declining as well. On flying insects, maximum concentrations of JAU 6476-desthio were reached slightly earlier, on DAT+1, at least for replicates 1 and 3 and declined rapidly.

The study provides measured field data on the time course of residue decline of spiroxamine, prothioconazole and JAU 6476-desthio in foliage-dwelling arthropods and flying insects.

I. Materials

Test Material	Input Classic® PTZ+SPX+ EC 160+300G
Lot/Batch #:	2013-002649
Purity:	300 g/L spiroxamine (nominal), 302.6 g/L (analyse)

	160 g/L prothioconazole (nominal), 161.5 g/L (analysed)
Description:	Not stated
Stability of test compound:	Stable at 25 ± 5°C, storage condition from +2°C to + 30°C also acceptable
Reanalysis/Expiry date:	5th June 2015
Density:	0.985 g/mL
Treatments	
Test rates:	Single application of 1.16 L/ha for replicate 1, 1.17 L/ha for replicate 2 and 1.14 L/ha for replicate 3
Solvent/vehicle:	Water was used as a carrier (300 L/ha)
Analysis of test concentrations:	Determined using high performance chromatography with mass spectrometry (HPLC-MS/MS)
Test design	
Test area:	Cereal fields (winter wheat) in the vicinity of Zuelpich, North Rhine-Westphalia in western Germany (3 fields of approximately 8.5, 4.8 and 2.8 ha, with replicates of approximately 1 ha per field). No fungicidal product was used before the start of the study. No insecticides were used during the growing season and field phase, BBCH 43-47.
Sampling:	Foliage-dwelling arthropods were collected before application (between DAT -2 and -1) and after application on DAT 0, +1, +2, +3, +5, +7 and +10, using gutters (10 cm × 200 cm) with an area of approximately 40 m² placed into the ground between the plant rows. A "knock down" insecticide was applied to enable collection after 1 to 2 hrs. Samples of flying insects were taken on the same days with the exception of DAT 0, using Malaise traps made of netting material (1mm mesh size) of dark colour at the bottom and white at the top (295 cm × 94 cm × 175 cm).
Duration of test:	10 days after application
Environmental test conditions	
Temperature:	Air temperature during field phase – 3.7 to 34.3°C Mean daily temperature – 1.1 to 26.5 °C (mean 17.9 °C) Minimum – 13 to 23°C
Rainfall:	During field phase – total: 15.0 mm. Daily rainfall: 0 – 8.5 mm

Study Design

The purpose of this study was to determine residue decline of spiroxamine, prothioconazole and JAU 6476-desthioin foliage-dwelling and flying arthropods following application with the formulated product Input Classic® (160 g/L Prothioconazole + 300 g/L Spiroxamine) at an application rate of 1.25 L product/ha in German cereal fields. The application was conducted at growth stage BBCH 43-47. Plant height ranged from 60 to 70 cm on day of application

Sampling schedule/methods

Sampling period was 10 days after application. Samples of foliage-dwellers were taken once before application (between DAT -2 to -1) and after the application on DAT 0 (approximately 4h after

application), +1, +2, +3, +5, +7, +10. Samples of flying insects were taken on the same days with the exception of DAT 0. Sampling areas were randomly assigned within the replicates.

Foliage-dwelling arthropods

Arthropods were collected into 100 gutters (10 cm × 200 cm) with an area of approximately 40 m² placed into the ground between plant rows.

To capture foliage-dwelling arthropods whole plants within defined areas of the cereal field were sprayed with a "knock down" insecticide (AquaPy®, active ingredient: natural pyrethrum, 30 g/L), applied at approximately 30 mL/L (non-GLP application). Approximately 0.5 L spray solution per 10 m³ cereal field was used to obtain a sample mass of ≥ 1.5 g. Approximately 1 to 2 hours after the application of the insecticide, all arthropds were collected from the gutters.

Flying insects

Samples of flying insects were collected using Malaise traps made of netting material (1mm mesh size) of dark colour at the bottom and white at the top (Bioform, 295 cm × 94 cm × 175 cm). Two traps per replicate were placed between the cereal plants close to the tram lines crossing the replicate. The trap was installed during the day and emptied after approximately 24 hours. Targeted biomass per sample was ≥ 1.5 g.

The composition of pooled arthropod samples was determined in terms of taxonomic groups, sub-divided into adults and larval stages. If larvae could not be distinguished from adults individuals were recorded as adults. Individuals of each groups were counted and the fresh weight of each taxonomic groups was determined.

Residue analysis

Samples were analysed for their content of spiroxamine, prothioconazole and its metabolite JAU 6476-desthio *via* HPLC-MS/MS. Residues were reported in terms of mg active substance/kg fresh weight (mg a.s./kg fw). The limit of quantification (LOQ) value was 0.01 mg/kg.

Daily temperature (minimum, maximum and mean) was obtained from the nearest official climate recording station in Nörvenich (approximately 16 km away from the study fields). Historical weather data was taken from climate station Bonn-Roleber (approximately 36km away from the fields). Daily rainfall measurements were taken with a rain gauge at the field laboratory (all field replicates were located inside a circumference of approximately 1.6 km around the field laboratory).

Analytical method

Samples of Insect were analysed using the validated analytical method M-529934-01-1, report reference M-529934-01-1 (**see Doc MCP Section 5**).

Statistics

For the residue decline of spiroxamine, prothioconazole and its metabolite JAU 6476-desthio in leaf dwelling arthropods and flying insects the 10-d TWA (time weighted average) was calculated by determination of the area under the decline curve. The residue per unit dose (RUD) was calculated with the actual application rate on each plot and the corresponding maximum residue concentration. The DT_{50} was determined to assess the time course of potential exposure of arthropods and in consequence of insectivorous birds. It was assumed that the residue decline followed a first-order kinetic.

II. Results and Discussion

The most dominant taxonomic groups within inventory samples and Malaise traps are presented in the tables below.

Table CP 10.1.1.2/30-1 Weight and abundance of arthropod groups (collected using inventory sampling)

Taxonomic group	Proportion of sampled biomass (%)	Biomass (g)
Lepidoptera larvae	63.88	33.65
Coleoptera	12.91	6.80
Arachnida	10.36	5.46
Hymenoptera	5.68	2.99
Aphidina	1.94	1.02
Coleoptera larvae	1.59	0.84
Dermaptera	1.27	0.67
Diptera	0.59	0.31
Lepidoptera	0.55	0.29
Opiliones	0.36	0.19
Heteroptera	0.30	0.16
Saltatoria	0.28	0.15
Saltatoria larvae	0.28	0.15
Acari	0.0	<0.01
Auchenorrhyncha	0.0	<0.01
Auchenorrhyncha larvae	0.0	<0.01
Formicidae	0.0	<0.01
Neuroptera	0.0	<0.01
Neuroptera larvae	0.0	<0.01

Table CP 10.1.1.2/30-2 Weight and abundance of arthropod groups (collected using Malaise trapping)

Taxonomic group	Proportion of sampled biomass (%)	Biomass (g)
Hymenoptera	69.33	10.85
Diptera	19.23	3.01
Lepidoptera	4.03	0.63
Arachnida	3.45	0.54
Coleoptera	3.45	0.54
Dermaptera	0.51	0.08
Aphidina	0.0	<0.01

Taxonomic group	Proportion of sampled biomass (%)	Biomass (g)
Auchenorrhyncha	0.0	<0.01
Formicidae	0.0	<0.01
Heteroptera	0.0	<0.01
Lepidoptera larvae	0.0	<0.01
Neuroptera	0.0	<0.01

Maximum residue values and the 10-day TWA for mean residue concentrations are presented in the table below. The residue decline of spiroxamine on leaf-dwelling arthropods and flying insects declined with initial fluctuations. Measured residue concentrations of prothioconazole rapidly declined after application in foliage-dwelling arthropods and flying insects. The metabolite JAU 6476-desthio reached its maximum concentrations on DAT+2 on foliage-dwellers before declining as well. On flying insects, maximum concentrations of JAU 6476-desthio were reached slightly earlier, on DAT+1, at least for replicates 1 and 3 and declined rapidly.

Table CP 10.1.1.2/30-3 Measured maximum residue, RUD and 10-d TWA concentrations on foliage dwelling invertebrates and flying insects

Compound	Matrix	Maximum residues (mg/kg) (RUD)			10-d time weighted average residues (mg/kg)		
		Repl. 1	Repl. 2	Repl. 3	Repl. 1	Repl. 2	Repl. 3
Spiroxamine	foliage dwellers	1.055 (3.03)	1.698 (4.84)	2.335 (6.83)	0.604	1.211	0.956
	flying insects	0.390 (1.12)	0.383 (1.09)	0.862 (2.52)	n.a.	0.147	0.264
Prothioconazole	foliage dwellers	0.305 (1.64)	0.368 (1.97)	0.356 (1.95)	0.077	0.091	0.109
	flying insects	0.110 (0.59)	0.098 (0.52)	0.313 (1.71)	n.a.	n.a.	0.057
JAU 6476-desthio	foliage dwellers	1.777 (9.58)	4.433 (23.68)	1.837 (10.07)	1.117	1.657	0.736
	flying insects	0.278 (1.50)	0.264 (1.41)	0.489 (2.68)	n.a.	0.080	0.121

n.a. = not available

RUD = residue per unit dose

Half-lives were calculated with residue values for spiroxamine, prothioconazole and its metabolite JAU 6476-desthio. Half-lives of prothioconazole and its metabolite were calculated independently because measured residue concentrations of JAU 6476-desthio were higher than those of the parent and the conversion could not be simulated, probably due to the very rapid dissipation of prothioconazole already before the first sampling. Results for DT_{50} simulations of residue decline in foliage-dwelling arthropods are presented below.

Table CP 10.1.1.2/30-4 DT_{50} of compounds on foliage-dwelling arthropods (SFO calculation)

Compound	DT_{50} (days) on flying insects		
	Repl. 1	Repl. 2	Repl. 3
Spiroxamine	5.0	5.6	2.9
Prothioconazole	1.3	1.2	0.9
JAU 6476-desthio	14.2	2.7	2.3

Table CP 10.1.1.2/30-5 DT_{50} of compounds on flying insects (SFO calculation)

Compound	DT_{50} (days) on flying insects		
	Repl. 1	Repl. 2	Repl. 3
Spiroxamine	2.9	1.5	2.0
Prothioconazole	1.7	1.9	0.8
JAU 6476-desthio	1.9	0.9	0.6

III. Conclusion

The study provides measured field data on the time course of residue decline of spiroxamine, prothioconazole and JAU 6476-desthio in foliage-dwelling arthropods and flying insects.

Assessment and conclusion by applicant:

There is no formal test guideline for the conduct of residues decline trials but some guidance is provided in the EFSA Guidance document on Risk Assessment for Birds and Mammals (2009) as well as the more recent EFSA Technical Report on general recurring issues in ecotoxicology (EFSA supporting publication 2019:EN-1673). Up to date regulatory intelligence on the expectations of the test design of such trials has also been taken in to consideration.

The study comprised one trial of three replicate winter cereal fields in Germany. The crop used was winter wheat which is highly relevant to the proposed representative GAP for cereals. The application rate was high enough in order to achieve sufficiently high residues of spiroxamine at the start of the trials, thereby allowing for a good decline curve to derive DT_{50} values from.

The analytical sampling regime adopted in the trials is considered to be suitable to derive reliable DT_{50} values, with sampling timepoints typically conducted on Days 0, 1, 2, 3, 5, 7 and 10. This regime is considered to meet the expectations for a robust residues decline trial, even with the relatively short expected DT_{50} for spiroxamine.

The sampling techniques adopted in the study are considered to have been adequate in order to take representative arthropod samples for residue analysis (knock-down sampling for foliar-dwelling arthropods and Malaise traps for flying insects). The sampling methods are considered to focus on foliar arthropods and flying insects. Soil-dwelling arthropods are not covered by these data.

Weather data were adequately recorded from the nearest weather station. Rainfall was measured at the study site and is therefore considered to be very accurate.

Overall, the data are considered reliable and suitable for inclusion in the derivation of refined DT_{50} values in cereals for use in Bird & Mammal risk assessment.

Data Point:	KCP 10.1.1.2/31
Report Author:	[redacted]; [redacted]
Report Year:	2016
Report Title:	Determination of the residues of prothioconazole, tebuconazole and spiroxamine in/on winter wheat after spray application of PTZ & SPX & TBZ EC 425 in northern France, Germany and the Netherlands
Report No:	16-2958
Document No:	M-574326-01-1
Guideline(s) followed in study:	Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market OECD Guideline for the Testing of Chemicals on Crop Field Trial (TG 509 published in September 2009) US EPA OCSPP 860.1500, Crop Field Trial
Deviations from current test guideline:	None
Previous evaluation:	No, not previously submitted -
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

Residues of prothioconazole, spiroxamine and tebuconazole were determined in/on winter wheat (green material) after one spray application with PTZ & SPX & TBZ EC 425, an emulsifiable concentrate formulation containing 53 g/L prothioconazole, 224 g/L spiroxamine and 148 g/L tebuconazole. The study included four supervised residue trials conducted in the field in Northern Europe (France, the Netherlands and two sites in Germany) during the 2016 season.

Average recoveries were within the range of 70-110%. No residues above the LOQ were found in control samples except for tebuconazole with a value of 0.11 mg/kg.

I. Materials

Test Material PTZ & SPX & TBZ EC 425

Lot/Batch #: ECE2102770

Purity: 53 g/L Prothioconazole (nominal); 50.54 g/L (analysed)
224 g/L Spiroxamine (nominal); 221.3 g/L (analysed)
148 g/L Tebuconazole (nominal); 149.7 g/L (analysed)

Description: Not stated

Stability of test compound: Not stated

Reanalysis/Expiry date: 10 February 2017

Density: Not stated

Treatments

Test rates: Single application consisting of 0.053 kg a.s./ha prothioconazole, 0.224 kg a.s./ha spiroxamine and 0.148 kg a.s./ha tebuconazole with a test item rate of 1.0 L/ha

Solvent/vehicle:	Water was used as a carrier (250-400 L/ha)
Analysis of test concentrations:	Determination of each of the actives and their associated metabolites was conducted using high performance liquid chromatography with mass spectrophotometric detection (HPLC-MS/MS).

Test design

Test area:	Four residue trials in northern France (clayey silt), Germany (sandy loam) and the Netherlands (clay). Each trial consisted of a treated and untreated plot. Plots ranged from 108 to 125 m^2
Sampling:	The sample material to be analysed was green material. Samples were collected on Day 0, 1, 2, 3, 6, 7 and 10 after last treatment (DALT) from the French study site, on Day 0, 1, 2, 3, 5, 7 and 10 from both German sites, and Day 0, 1, 2, 3, 5, 7 and 10 from the Netherlands site.
Duration of test:	10 days

Environmental test conditions

Temperature:	During application – 5.0 to 25.0 °C
Relative humidity:	During application – 54.2 to 73 %
pH:	Soil pH in water - 6.6 in Germany, 8.4 in France Soil pH in CaCl – 5.4 in Germany Soil pH in KCl – 7.5 in the Netherlands

Study Design

The objective of this study was to determine the magnitude of the residues of prothioconazole (comprising prothioconazole and its metabolite JAU 6476-desthio), spiroxamine and tebuconazole in/on winter wheat (BBCH 29-31, trial dependant) after one spray application with PTZ & SPX & TBZ EC 425. The study measured residues immediately following a single application (consisting of 0.053 kg a.s./ha prothioconazole, 0.224 kg a.s./ha spiroxamine and 0.148 kg a.s./ha tebuconazole with a test item rate of 1.0 L/ha) and up to 10 days later. The study included four supervised residue trials conducted in the field in Northern Europe (France, two sites in Germany and the Netherlands), with plots ranging from 108 to 125 m^2. At each trial site there was one untreated plot in addition to the treated plot(s). The treated and untreated plots were cultivated in the same manner

Sprayers were calibrated before each application and water was used as a carrier at a rate of between 250 and 400 L/ha, trial dependant.

The sample material to be analysed as green material. Analysis was conducted using HPLC-MS/MS.

Climatic and irrigation data were recorded (not according to GLP) during the conduct of the field trials.

Analytical method

Samples of wheat green material were analysed using the validated analytical method 01089, report reference M-304677-01-1 (**see Doc MCP Section 5**).

H. Results and Discussion

The study was deemed to be acceptable based on the criteria set out in the US EPA OCSPP 860.1500, Crop Field Trial (1996) and OECD guideline 509 for The testing of Chemicals on Crop Field Trial (2009).

Mean temperatures ranged from 3 to 8°C in the French trial (16-2958-01), 6 to 13°C in the German trial (16-2958-02), 8 to 12°C in the Netherlands trial (16-2958-03) and 6 to 14°C in the other German trial (16-2958-04). Rainfall ranged from 0 to 10 mm in France, 0 to 4 mm in Germany, 0 to 4 mm in the Netherlands and 0 to 6 mm in the second German trial. No irrigation was applied in any of the four trials.

The application rate of prothioconazole, spiroxamine and tebuconazole for each trial was 0.053, 0.224 and 0.148 kg a.s./ha, respectively.

The residue levels determined in the treated samples are summarised in the tables below;

Table CP 10.1.1.2/31-1 Measured residues of Prothioconazole, JAU 6476-desthio, tebuconazole and spiroxamine in/on winter wheat

Country	BBCH growth stage	DALT	Residues (mg/kg)			
			prothioconazole		tebuconazole	spiroxamine
			Prothioconazole	JAU 6476-desthio		
France (16-2958-01)	29	0	0.70	2.5	16	16
	29	1	0.059	0.26	3.7	2.8
	29	2	0.043	0.19	3.3	2.2
	29	3	0.040	0.18	3.6	2.3
	30	6	0.024	0.11	2.9	1.3
	30	7	0.018	0.089	2.6	1.1
	30	10	0.012	0.063	2.1	0.66
Germany (16-2958-02)	29	0	0.44	1.4	10	8.8
	29	1	0.10	1.6	9.2	6.5
	29	2	0.049	1.2	8.2	5.4
	30	4	0.033	0.82	7.9	4.9
	30	5	0.010	0.25	3.8	2.1
	30	7	0.01	0.15	3.4	1.6
	30	10	<0.01	0.066	2.7	1.0
The Netherlands (16-2958-03)	29	0	0.48	1.4	9.9	9.4
	29	1	0.050	0.39	4.3	3.6
	30	2	0.055	0.31	4.5	3.3
	30	3	0.049	0.22	3.8	2.7
	30	5	0.026	0.12	3.3	1.5
	30	7	0.019	0.085	2.6	1.0
	31	10	<0.01	0.043	1.6	0.53
Germany (16-2958-04)	29	0	0.47	1.0	7.4	7.3
	29	1	0.043	0.29	2.5	3.6
	30	2	0.025	0.18	2.3	2.9
	30	3	0.021	0.11	2.3	2.6

Country	BBCH growth stage	DALT	Residues (mg/kg)			
			prothioconazole		tebuconazole	spiroxamine
			Prothioconazole	JAU 6476-desthio		
	30	5	0.014	0.061	1.8	2.0
	31	7	0.012	0.047	1.6	1.5
	31	10	<0.01	0.027	1.2	1.1

DALT = Days after last application

Table CP 10.1.1.2/31-2 Summmary of measured residues in/on winter wheat after application of PTZ & SPX & TBZ EC 425

Analyte	BBCH growth stage	DALT	Residues (mg/kg)
prothioconazole	29	0	0.44-0.70
		1	0.043-0.10
	29-30	2	0.025-0.055
		3	0.021-0.049
	30	5	0.010-0.026
		6	0.024
	30-31	7	<0.01-0.019
		10	<0.01-0.012
JAU 6476-desthio	29	0	1.0-2.5
		1	0.26-1.6
	29-30	2	0.18-1.2
		3	0.11-0.82
	30	5	0.061-0.25
		6	0.11
	30-31	7	0.047-0.15
		10	0.027-0.066
spiroxamine	29	0	7.3-16
		1	2.8-6.5
	29-30	2	2.2-5.4
		3	2.3-4.9
	30	5	1.5-2.1
		6	1.3
	30-31	7	1.0-1.7
		10	0.53-1.1
tebuconazole	29	0	7.4-16

Analyte	BBCH growth stage	DALT	Residues (mg/kg)
		1	2.5-9.2
	29-30	2	2.3-8.2
		3	2.3-7.9
	30	5	1.8-3.8
		6	2.9
	30-31	7	1.6-3.4
		10	1.2-2.7

DALT = Days after last application

III. Conclusion

Average recoveries were within the range of 70-110%. No residues above the LOQ were found in control samples, except for tebuconazole with a value of 0.11 mg/kg.

Assessment and conclusion by applicant:

There is no formal test guideline for the conduct of residues decline trials but some guidance is provided in the EFSA Guidance document on Risk Assessment for Birds and Mammals (2009) as well as the more recent EFSA Technical Report on general recurring issues in ecotoxicology (EFSA supporting publication 2019:EN-1673). Up to date regulatory intelligence on the expectations of the test design of such trials has also been taken in to consideration.

The study comprised four trials over three countries in NEU (Germany, the Netherlands and Northern France). The crop used was winter wheat which is highly relevant to the proposed representative GAP for cereals. The application rate was high enough in order to achieve sufficiently high residues of spiroxamine at the start of the trials, thereby allowing for a good decline curve to derive DT_{50} values from.

The analytical sampling regime adopted in the trials is considered to be suitable to derive reliable DT_{50} values, with sampling timepoints typically conducted on Days 0, 1, 2, 3, 5, 7 and 10. This regime is considered to meet the expectations for a robust residues decline trial, even with the relatively short expected DT_{50} for spiroxamine.

Weather data were adequately recorded from the nearest weather station.

Overall, the data are considered reliable and suitable for inclusion in the derivation of refined DT_{50} values in cereals for use in Bird & Mammal risk assessment.

Report M-759383-01-1 presents the results of the kinetic modeling for the spiroxamine data measured for these trials as well as the other available decline studies on cereals. An overall DT_{50} value for spiroxamine has been determined using all of these data. As part of the analysis, trials that measured >1 mm rainfall in the first 24 hours were both included and excluded in order to assess the impact that this rainfall may have had on the overall DT_{50} value. It was concluded that there was very little variation in the mean DT_{50} value when these trials were either included or excluded. Thus, it is considered that any rainfall recorded in these trials has not adversely affected the results achieved therefore these trials are considered to be valid and can be included in the determination of the residue decline for spiroxamine.

Data Point:	KCP 10.1.1.2/32
Report Author:	[redacted]
Report Year:	2017
Report Title:	Determination of the residues of prothioconazole, tebuconazole and spiroxamine in/on winter wheat after spray application of PTZ & SPX & TBZ EC 425 in southern France, Spain, Italy and Portugal
Report No:	16-2952
Document No:	M-578235-01-1
Guideline(s) followed in study:	Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market OECD Guideline for the Testing of Chemicals on Crop Field Trial (TG 509 published in September 2009) US EPA OCSPP 860.1500, Crop Field Trial
Deviations from current test guideline:	None
Previous evaluation:	No, not previously submitted -
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

Residues of prothioconazole, spiroxamine and tebuconazole were determined in/on wheat (green material) after one spray application with PTZ & SPX & TBZ EC 425, an emulsifiable concentrate formulation containing 53 g/L prothioconazole, 224 g/L spiroxamine and 148 g/L tebuconazole. The study included four supervised residue trials conducted in the field in Southern Europe (France, Spain, Italy and Portugal) during the 2016 season.

Average recoveries were within the range of 70-110%. No residues above the LOQ were found in control samples.

I. Materials

Test Material PTZ & SPX & TBZ EC 425

Lot/Batch #: ECE2102770

Purity: 53 g/L Prothioconazole (nominal); 50.54 g/L (analysed)
224 g/L Spiroxamine (nominal); 221.3 g/L (analysed)
148 g/L Tebuconazole (nominal); 149.7 g/L (analysed)

Description: Not stated

Stability of test compound: Not stated

Reanalysis/Expiry date: 10 February 2017

Density: Not stated

Treatments

Test rates: Single application consisting of 0.053 kg a.s./ha prothioconazole, 0.224 kg a.s./ha spiroxamine and 0.148 kg a.s./ha tebuconazole with a test item rate of 1.0 L/ha

Solvent/vehicle: Water was used as a carrier (300-400 L/ha)

Analysis of test concentrations:	Determination of each of the actives and their associated metabolites was conducted using high performance liquid chromatography with mass spectrophotometric detection (HPLC-MS/MS).
Test design	
Test area:	Four residue trials in southern France (silty clay), Spain (clay), Italy (sandy loam) and Portugal (clay). Each trial consisted of a treated and untreated plot. Plots ranged from 45 to 87.5 m^2
Sampling:	The sample material to be analysed was green material. Samples were collected on Day 0, 1, 2, 3, 5, 7 and 9 after last treatment (DALT) from the French study site, on Day 0, 1, 2, 4, 5, 7 and 10 from the Spanish site, Day 0, 1, 2, 3, 4, 8 and 10 from the Italian site and Day 0, 1, 2, 3, 5, 7 and 10 from the Portuguese site.
Duration of test:	10 days (9 for France)
Environmental test conditions	
Temperature:	During application – 8.0 to 15.0°C
Relative humidity:	During application – 67.5 to 81 %
pH:	6.9 to 8.3 (soil pH in water)

Study Design

The objective of this study was to determine the magnitude of the residues of prothioconazole (comprising prothioconazole and its metabolite JAU 6476-desthio), spiroxamine and tebuconazole in/on winter wheat (BBCH 23-30, trial dependant) after one spray application with PTZ & SPX & TBZ EC 425. The study measured residues immediately following a single application (consisting of 0.053 kg a.s./ha prothioconazole, 0.224 kg a.s./ha spiroxamine and 0.148 kg a.s./ha tebuconazole with a test item rate of 1.0 L/ha) and up to 10 days later (with the exception of France where the last sampling was 9 DALT). The study included four supervised residue trials conducted in the field in Southern Europe (France, Spain, Italy and Portugal), with plots ranging from 45 to 87.5 m^2. Sprayers were calibrated before each application and water was used as a carrier at a rate of between 200 and 400 L/ha, trial dependant.

The sample material to be analysed as green material. Analysis was conducted using HPLC-MS/MS.

Climatic and irrigation data were recorded (not according to GLP) during the conduct of the field trials.

Analytical method

Samples of wheat/green material were analysed using the validated analytical method 01089, report reference M-304677-01-1 (**see Doc MCP Section 5**).

II. Results and Discussion

The study was deemed to be acceptable based on the criteria set out in the US EPA OCSPP 860.1500, Crop Field Trial (1996) and OECD guideline 509 for The testing of Chemicals on Crop Field Trial (2009).

Mean temperatures ranged from 6 to 12°C in the French trial (16-2952-01), 6 to 13°C in the Spanish trial (16-2952-02), 9 to 15°C in the Italian trial (16-2952-03) and between 11 and 12°C in the Portuguese trial (16-2952-04). Rainfall ranged from 0 to 15 mm in France, 0 mm in Spain, 0 mm in Italy and 0 to 15 mm in Portugal. No irrigation was applied in any of the four trials.

The application rate of prothioconazole, spiroxamine and tebuconazole for each trial was 0.053, 0.224 and 0.148 kg a.s./ha, respectively.

The residue levels determined in the treated samples are summarised in the tables below;

Table CP 10.1.1.2/32-1 Measured residues of Prothioconazole, JAU 6476-desthio, tebuconazole and spiroxamine in/on winter wheat

Country	BBCH growth stage	DALT	Residues (mg/kg)			
			prothioconazole		tebuconazole	spiroxamine
			Prothioconazole	JAU 6476-desthio		
France (16-2952-01)	29	0	0.46	2.3	13	16
	29	1	0.10	0.78	8.6	7.1
	29	2	0.065	0.36	5.6	4.9
	29	3	0.050	0.27	5.5	4.3
	29	5	0.030	0.13	3.8	2.9
	30	7	0.018	0.066	3.1	2.1
	30	9	0.014	0.031	1.7	1.5
Spain (16-2952-02)	23	0	0.44	1.6	9.6	12
	23	1	0.24	1.2	11	6.1
	25	2	0.17	1.1	11	4.9
	25	4	0.046	0.54	5.1	3.4
	25	5	0.035	0.41	4.3	2.5
	29	7	0.032	0.42	4.7	2.4
	30	10	0.020	0.28	3.9	1.6
Italy (16-2952-03)	30	0	0.37	0.99	7.5	7.7
	30	1	0.11	0.43	4.5	4.3
	31	2	0.078	0.46	4.6	4.3
	31	3	0.042	0.39	3.6	3.8
	31	4	0.027	0.31	3.7	3.4
	32	8	0.011	0.11	3.4	2.4
	32	10	<0.01	0.046	2.3	1.6
Portugal (16-2952-04)	29	0	0.47	1.5	7.9	9.8
	29	1	0.039	0.35	3.8	4.6
	29	2	0.037	0.34	3.5	4.4
	29	3	0.035	0.33	3.0	4.6
	29	5	0.015	0.084	2.0	2.8
	29	7	0.011	0.057	2.0	2.4
	30	10	<0.01	0.033	1.6	1.9

DALT = Days after last application

Table CP 10.1.1.2/32-2 Summmary of measured residues in/on winter wheat after application of PTZ & SPX & TBZ EC 425

Analyte	BBCH growth stage	DALT	Residues (mg/kg)
prothioconazole	23-30	0	0.37-0.47
		1	0.039-0.24
	25-31	2	0.037-0.17
	25-31	3-4	0.035-0.050
	25-31	4-5	0.015-0.035
	29-32	7-8	0.011-0.032
	30-32	9-10	0.01-0.020
JAU 6476-desthio	23-30	0	0.97-2.3
		1	0.35-1.2
	25-31	2	0.24-1.1
	25-31	3-4	0.27-0.54
	25-31	4-5	0.084-0.41
	29-32	7-8	0.057-0.42
	30-32	9-10	0.031-0.28
tebuconazole	23-30	0	7.5-13
		1	3.8-11
	25-31	2	3.5-11
	25-31	3-4	3.0-5.5
	25-31	4-5	2.0-4.3
	29-32	7-8	2.0-4.7
	30-32	9-10	1.6-3.9
spiroxamine	23-30	0	7.7-16
		1	4.3-7.1
	25-31	2	4.3-4.9
	25-31	3-4	3.4-4.6
	25-31	4-5	2.5-3.4
	29-32	7-8	2.1-2.4
	30-32	9-10	1.5-1.9

DALT = Days after last application

III. Conclusion

Average recoveries were within the range of 70-110%. No residues above the LOQ were found in control samples.

Assessment and conclusion by applicant:

There is no formal test guideline for the conduct of residues decline trials but some guidance is provided in the EFSA Guidance document on Risk Assessment for Birds and Mammals (2009) as well as the more recent EFSA Technical Report on general recurring issues in ecotoxicology (EFSA supporting publication 2019:EN-1673). Up to date regulatory intelligence on the expectations of the test design of such trials has also been taken in to consideration.

The study comprised four trials over four countries in SEU (France, Spain, Italy and Portugal). The crop used was winter wheat which is highly relevant to the proposed representative GAP for cereals. The application rate was high enough in order to achieve sufficiently high residues of spiroxamine at the start of the trials, thereby allowing for a good decline curve to derive DT_{50} values from.

The analytical sampling regime adopted in the trials is considered to be suitable to derive reliable DT_{50} values, with sampling timepoints typically conducted on Days 0, 1, 2, 3, 5, 7 and 10. This regime is considered to meet the expectations for a robust residues decline trial, even with the relatively short expected DT_{50} for spiroxamine.

Weather data were adequately recorded from the nearest weather station.

Overall, the data are considered reliable and suitable for inclusion in the derivation of refined DT_{50} values in cereals for use in Bird & Mammal risk assessment.

Report M-759383-01-1 presents the results of the kinetic modeling for the spiroxamine data measured for these trials as well as the other available decline studies on cereals. An overall DT_{50} value for spiroxamine has been determined using all of these data. As part of the analysis, trials that measured >1 mm rainfall in the first 24 hours were both included and excluded in order to assess the impact that this rainfall may have had on the overall DT_{50} value. It was concluded that there was very little variation in the mean DT_{50} value when these trials were either included or excluded. Thus, it is considered that any rainfall recorded in these trials has not adversely affected the results achieved therefore these trials are considered to be valid and can be included in the determination of the residue decline for spiroxamine. It is acknowledged that there was no rainfall recorded during the trials in Spain and Italy.

Data Point:	KCP 10.1.1.2/33
Report Author:	[redacted]
Report Year:	2018
Report Title:	Amendment no. 1 to final report - Determination of the residues of prothioconazole, spiroxamine and trifloxystrobin in/on wheat after spray application of PTZ & SPX & TFS EC 280.3 in Germany, northern France, the Netherlands and Belgium
Report No:	17-2950
Document No:	M-628347-02-1
Guideline(s) followed in study:	Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market OECD Guideline for the Testing of Chemicals on Crop Field Trial (TG 509 published in September 2009) US EPA OCSPP 860.1500, Crop Field Trial
Deviations from current test guideline:	None
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

Residues of prothioconazole, sprioxamine and trifloxystrobin were determined in/on wheat (green material) after one spray application with PTZ & SPX & TFS EC 280.3, an emulsifiable concentrate formulation containing 93.3g/L prothioconazole, 107 g/L spiroxamine and 80 g/L trifloxystrobin. The study included four supervised residue trials conducted in the field in Northern Europe (Germany, Northern France, the Netherlands and Belgium).

Residues of prothioconazole comprised of prothioconazole and its metabolite JAU 6476-desthio. Residues of sprioxamine comprised of the spiroxamine enantiomers A1, A2, B1 and B2, the total residue of the parent spiroxamine as the sum of the four enantiomers. Residues of trifloxystrobin comprising trifloxystrobin and its isomers/metabolites CGA 331409, CGA 357262, CGA 357261, CGA 321013 and CGA 373466.

Average recoveries were within the range of 70-110%. No residues above the LOQ were found in control samples.

I. Materials

Test Material	PTZ & SPX & TFS EC 280.3
Lot/Batch #:	201-001477
Purity:	93.3 g/L Prothioconazole (nominal); 92.40 g/L (analysed) 107 g/L Spiroxamine (nominal); 107.8 g/L (analysed) 80 g/L Trifloxystrobin (nominal); 79.55 g/L (analysed)
Description:	Not stated
Stability of test compound:	Not stated
Reanalysis/Expiry date:	21 March 2017
Density:	Not stated
Treatments	
Test rates:	Single application consisting of 0.140 kg a.s./ha prothioconazole, 0.161 kg a.s./ha spiroxamine and 0.120 kg a.s./ha trifloxystrobin with a test item rate of 1.5 L/ha
Solvent/vehicle:	Water was used as a carrier (100-350 L/ha)
Analysis of test concentrations:	Determination of each of the actives and their associated metabolites was conducted using high performance liquid chromatography with mass spectrophotometric detection (HPLC-MS/MS).
Test design	
Test area:	Four residue trials in Germany (sandy loam), Northern France (clayey silt), the Netherlands (clay) and Belgium (silt loam). Each trial consisted of a treated and untreated plot. Plots ranged from 100 to 156 m^2
Sampling:	The sample material to be analysed was green material. Samples were collected on Day -0, 0, 1, 2, 3, 5, 7 and 10 after last treatment (DALT) from each of the four study sites.
Duration of test:	10 days (11 for Belgium)

Environmental test conditions

Temperature:	During application – 12.0 to 19.0°C
Relative humidity:	During application – 40 to 73%
pH:	6.7 to 8.1 (soil pH)

Study Design

The objective of this study was to determine the magnitude of the residues of prothioconazole (comprising prothioconazole and its metabolite JAU 6476-desthio), the residues of spiroxamine (comprising the spiroxamine enantiomers A1, A2, B1 and B2, the total residue of parent spiroxamine as the sum of the four enantiomers) and trifloxystrobin (comprising trifloxystrobin and its isomers/metabolites CGA 331409, CGA 357262, CGA 357261, CGA 321113 and CGA 373466) in/on wheat (BBCH 30) after one spray application with PTZ & SPX & TFS EC 280+3. The study measured residues immediately following a single application (consisting of 0.140 kg a.s./ha prothioconazole, 0.161 kg a.s./ha spiroxamine and 0.120 kg a.s./ha trifloxystrobin 400 g a.s./ha with a test item rate of 1.5 L/ha) and up to 10 days later (with the exception of Belgium where the last sampling was 11 DALT). The study included four supervised residue trials conducted in the field in Northern Europe (Germany, Northern France, the Netherlands and Belgium), with plots ranging from 100 to 156 m². Sprayers were calibrated before each application and water was used as a carrier at a rate of between 100 and 350 L/ha, trial dependant.

The sample material to be analysed was green material. Analysis was conducted using HPLC-MS/MS. For the control sample taken at -0 DALT, the total green material sample amount of 25 plants was weighed and recorded in order to obtain an approximate single plant weight. The weight of 25 plants in Germany, Northern France, The Netherlands and Belgium was 53 g, 63.5 g, 165 g and 792 g, respectively.

Climatic and irrigation data were recorded (without GLP) during the conduct of the field trials.

Analytical method

Samples of wheat/green material were analysed using the validated analytical method 01480, report reference M-628347-02-1 (**see Doc MCP Section 5**).

II. Results and Discussion

The study was deemed to be acceptable based on the criteria set out in the US EPA OCSPP 860.1500, Crop Field Trial (1996) and OECD guideline 509 for the testing of Chemicals on Crop Field Trial (2009).

Mean temperatures ranged from 8 to 16°C in the German trial (17-2950-01), 6 to 15°C in the Northern France trial (17-2950-02), 14 to 19°C in the Netherland trial (17-2950-03) and between 12 and 23°C in the Belgium trial (17-2950-04). Rainfall ranged from 0 to 3 mm in the German trial, 0 to 12 mm in Northern France, 0 to 21 mm in the Netherlands and 0 to 7 mm in Belgium. No irrigation was applied in any of the four trials.

The application rate of prothioconazole, spiroxamine and trifloxystrobin for each trial was 0.140, 0.161 and 0.120 kg a.s./ha, respectively.

The residue levels determined in the treated samples are summarised in the tables below;

Table CP 10.1.1.2/33-1 Measured residues of prothioconazole and JAU 6476-desthio in/on wheat

Country	BBCH growth stage	DALT	Residues (mg/kg)	
			a.s. prothioconazole	
			Prothioconazole	JAU 6476-desthio
Germany (17-2950-01)	30	0	2.3	4.2
	30	1	0.26	3.4
	30	2	0.11	2.2
	30	3	0.10	2.0
	30	5	0.085	1.2
	30	7	0.067	0.75
	30	10	0.077	0.37
Northern France (17-2950-02)	30	0	2.3	4.3
	30	1	0.13	0.62
	30	2	0.10	0.58
	30	3	0.042	0.28
	30	5	0.043	0.20
	31	7	0.028	0.084
	31	10	0.020	0.036
The Netherlands (17-2950-03)	30	0	1.6	2.9
	30	1	0.15	2.8
	31	2	0.062	2.4
	31	3	0.046	2.1
	32	5	0.024	1.4
	32	7	0.015	0.73
	39	10	0.011	0.31
Belgium (17-2950-04)	30	0	3.1	7.4
	30	1	0.63	5.4
	30	2	0.12	4.8
	31	3	0.023	2.6
	31	5	0.015	1.6
	32	7	0.011	0.89
	37	11	<0.01	0.24

DALT = Days after last application

Table CP 10.1.1.2/33-2 Measured residues of spiroxamine and its enantiomers in/on wheat

Country	BBCH growth stage	DALT	Residues (mg/kg)				
			a.s. spiroxamine				
			KWG 4168-A1 enantiomer	KWG 4168-A2 enantiomer	KWG 4168-B1 enantiomer	KWG 4168-B2 enantiomer	Total residue of 4 enantiomers
Germany (17-2950-01)	30	0	1.7	1.7	1.4	1.3	6.1
	30	1	0.94	0.92	0.75	0.70	3.3
	30	2	0.57	0.55	0.45	0.44	2.0
	30	3	0.53	0.53	0.42	0.40	1.9
	30	5	0.43	0.42	0.32	0.33	1.5
	30	7	0.28	0.28	0.23	0.22	1.0
	30	10	0.22	0.21	0.17	0.17	0.78
Northern France (17-2950-02)	30	0	1.9	1.8	1.5	1.4	6.5
	30	1	0.31	0.30	0.26	0.25	1.1
	30	2	0.30	0.29	0.25	0.26	1.1
	30	3	0.20	0.20	0.19	0.18	0.77
	30	5	0.18	0.19	0.17	0.17	0.71
	31	7	0.12	0.12	0.11	0.11	0.45
	30	10	0.074	0.076	0.067	0.064	0.28
The Netherlands (17-2950-03)	30	0	1.0	1.0	0.82	0.79	3.6
	30	1	0.54	0.52	0.41	0.40	1.9
	31	2	0.47	0.45	0.36	0.36	1.6
	31	3	0.40	0.40	0.31	0.31	1.4
	32	5	0.25	0.24	0.19	0.20	0.88
	32	7	0.18	0.18	0.15	0.14	0.65
	39	10	0.10	0.10	0.086	0.082	0.37
Belgium (17-2950-04)	30	0	1.7	1.7	1.3	1.3	6.0
	30	1	0.98	0.97	0.80	0.77	3.5
	30	2	0.74	0.75	0.61	0.60	2.7
	31	3	0.44	0.42	0.34	0.35	1.6
	31		0.25	0.25	0.20	0.21	0.91
	32	7	0.20	0.20	0.18	0.17	0.75
	37	11	0.083	0.082	0.070	0.070	0.30

DALT = Days after last application

Table CP 10.1.1.2/33-3 Measured residues of trifloxystrobin and metabolites/isomers in/on wheat

Country	BBCH growth stage	DALT	Residues (mg/kg)					
			a.s. trifloxystrobin					
			trifloxystrobin	CGA 331409	CGA 357262	CGA 357261	CGA 321113	CGA 373466
Germany (17-2950-01)	30	0	8.3	0.071	<0.01	0.17	0.14	<0.01
	30	1	5.5	0.17	0.027	0.38	0.35	0.014
	30	2	2.1	0.20	0.10	0.53	0.16	0.015
	30	3	1.6	0.23	0.17	0.57	0.13	0.031
	30	5	1.2	0.25	0.23	0.61	0.058	0.014
	30	7	0.56	0.17	0.10	0.34	0.032	<0.01
	30	10	0.39	0.11	0.13	0.22	0.022	<0.01
Northern France (17-2950-02)	30	0	8.2	0.035	<0.01	0.063	0.46	<0.01
	30	1	1.6	0.089	0.005	0.11	0.14	<0.01
	30	2	1.5	0.13	0.041	0.22	0.12	0.010
	30	3	0.35	0.054	0.019	0.060	0.057	<0.01
	30	5	0.32	0.060	0.031	0.092	0.023	<0.01
	31	7	0.15	0.042	0.035	0.068	<0.01	<0.01
	31	10	0.075	0.023	0.022	0.033	<0.01	<0.01
The Netherlands (17-2950-03)	30	0	5.6	0.055	<0.01	0.10	0.092	<0.01
	30	1	2.8	0.17	0.056	0.38	0.17	0.021
	31	2	3.0	0.26	0.17	0.65	0.16	0.029
	31	3	1.9	0.23	0.15	0.49	0.095	0.027
	32	5	0.94	0.18	0.15	0.28	0.043	0.013
	37	7	0.64	0.12	0.11	0.15	0.015	<0.01
	39	10	0.076	0.049	0.047	0.013	<0.01	<0.01
Belgium (17-2950-04)	30	0	10	0.31	0.023	0.58	0.34	0.026
	30	1	6.0	0.51	0.14	0.95	0.23	0.041
	30	2	2.9	0.57	0.31	1.0	0.30	0.096
	31	3	0.32	0.25	0.17	0.12	0.073	0.015
	31	5	0.094	0.15	0.11	0.035	0.026	<0.01
	32	7	0.047	0.080	0.069	0.027	0.010	<0.01
	37	11	<0.01	0.018	0.022	<0.01	<0.01	<0.01

DALT = Days after last application

III. Conclusion

Residues of prothioconozole comprised of prothioconazole and its metabolite JAU 6476-desthio. Residues of sprioxamine comprised of the spiroxamine enantiomers A1, A2, B1 and B2, the total residue of the parent spiroxamine as the sum of the four enantiomers. Residues of trifloxystrobin comprising trifloxystrobin and its isomers/metabolites CGA 331409, CGA 357262, CGA 357261, CGA 321113 and

CGA 373466.

Average recoveries were within the range of 70-110%. No residues above the LOQ were found in control samples.

Assessment and conclusion by applicant:

There is no formal test guideline for the conduct of residues decline trials but some guidance is provided in the EFSA Guidance document on Risk Assessment for Birds and Mammals (2009) as well as the more recent EFSA Technical Report on general recurring issues in ecotoxicology (EFSA supporting publication 2019:EN-1673). Up to date regulatory intelligence on the expectations of the test design of such trials has also been taken in to consideration.

The study comprised four trials over four countries in NEU (Germany, Northern France, the Netherlands and Belgium). The crop used was wheat which is highly relevant to the proposed representative GAP for cereals. The application rate was high enough in order to achieve sufficiently high residues of spiroxamine at the start of the trials, thereby allowing for a good decline curve to derive DT_{50} values from.

The analytical sampling regime adopted in the trials is considered to be suitable to derive reliable DT_{50} values, with sampling timepoints typically conducted on Days 0, 1, 2, 3, 5, 7 and 10. This regime is considered to meet the expectations for a robust residues decline trial, even with the relatively short expected DT_{50} for spiroxamine.

Weather data were adequately recorded from the nearest weather station.

Overall, the data are considered reliable and suitable for inclusion in the derivation of refined DT_{50} values in cereals for use in Bird & Mammal risk assessment.

Report M-759383-01-1 presents the results of the kinetic modeling for the spiroxamine data measured for these trials as well as the other available decline studies on cereals. An overall DT_{50} value for spiroxamine has been determined using all of these data. As part of the analysis, trials that measured >1 mm rainfall in the first 24 hours were both included and excluded in order to assess the impact that this rainfall may have had on the overall DT_{50} value. It was concluded that there was very little variation in the mean DT_{50} value when these trials were either included or excluded. Thus, it is considered that any rainfall recorded in these trials has not adversely affected the results achieved therefore these trials are considered to be valid and can be included in the determination of the residue decline for spiroxamine.

Data Point:	KCP 10.1.1.2/34
Report Author:	[redacted]; [redacted]
Report Year:	2020
Report Title:	Determination of the residues of trifloxystrobin, prothioconazole and spiroxamine in/on wheat after spray application of PTZ & SPX & TFS EC 280.3 in the field in Germany, Belgium and the Netherlands - Final report -
Report No:	E19RP088
Document No:	M-684671-01-1
Guideline(s) followed in study:	Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market OECD Guideline for the Testing of Chemicals on Crop Field Trial (TG 509 published in September 2009) US EPA OCSPP 860.1500, Crop Field Trial
Deviations from current test guideline:	None
Previous evaluation:	No, not previously submitted -
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The purpose of the study E19RP088 was to determine the magnitude of the residues of prothioconazole (comprising prothioconazole and its degradation product JAU 6476-desthio), spiroxamine (comprising KWG 4168-A1 enantiomer, KWG 4168-A2 enantiomer, KWG 4168-B1 enantiomer, KWG 4168-B2 enantiomer, the total residue of spiroxamine parent as the sum of the four enantiomers, and the total residue of spiroxamine (*via* 4-tbutylcyclohexanone)), the residues of trifloxystrobin (comprising trifloxystrobin, CGA 331409, CGA 357262, CGA 357261, CGA 321113 and CGA 373466) in/on wheat (green material) after one spray application with PTZ & SPX & TFS EC 280.3, an emulsifiable concentrate (EC) formulation containing 93.3 g/L prothioconazole, 107 g/L spiroxamine and 80 g/L trifloxystrobin.

I. Materials

Test Material PTZ & SPX & TFS EC 280.3

Lot/Batch #: 201-001477

Purity: 93.3 g/L Prothioconazole (nominal); 90.42 g/L (analysed)
107 g/L Spiroxamine (nominal); 107.4 g/L (analysed)
80 g/L Trifloxystrobin (nominal); 73.83 g/L (analysed)

Description: Not stated

Stability of test compound: Not stated

Reanalysis/Expiry date: 22 March 2022

Density: Not stated

Treatments

Test rates:	E19RP088-01, Germany: Single application consisting of 0.142 kg a.s./ha prothioconazole, 0.163 kg a.s./ha spiroxamine and 0.122 kg a.s./ha trifloxystrobin with a test item rate of 1.52 L/ha E19RP088-02, Germany: Single application consisting of 0.150 kg a.s./ha prothioconazole, 0.172 kg a.s./ha spiroxamine and 0.129 kg a.s./ha trifloxystrobin with a test item rate of 1.61 L/ha E19RP088-03, Belgium: Single application consisting of 0.140 kg a.s./ha prothioconazole, 0.161 kg a.s./ha spiroxamine and 0.120 kg a.s./ha trifloxystrobin with a test item rate of 1.61 L/ha E19RP088-04, Netherlands: Single application consisting of 0.139 kg a.s./ha prothioconazole, 0.160 kg a.s./ha spiroxamine and 0.120 kg a.s./ha trifloxystrobin with a test item rate of 1.49 L/ha
Solvent/vehicle:	Water was used as a carrier (E19RP088-01, Germany: 306 L/ha, E19RP088-02, Germany: 322 L/ha, E19RP088-03, Belgium: 301 L/ha, E19RP088-04, Netherlands: 299 L/ha.
Analysis of test concentrations:	Determination of each of the actives and their associated metabolites was conducted using high performance liquid chromatography with mass spectrophotometric detection (HPLC-MS/MS).

Environmental test conditions

Temperature:	E19RP088-01 = 7.0 to 14.0°C, E19RP088-02 = 12 to 20°C, E19RP088-03 = 9.0 to 12.0°C and E19RP088-04 = 8.0 to 17.0°C
Relative humidity:	Not stated
pH:	Not stated

Study design

The purpose of the study E19RP088 was to determine the magnitude of the residues of prothioconazole (comprising prothioconazole and its degradation product JAU 6476-desthio), spiroxamine (comprising KWG 4168-A1 enantiomer, KWG 4168-A2 enantiomer, KWG 4168-B1 enantiomer, KWG 4168-B2 enantiomer, the total residue of spiroxamine parent as the sum of the four enantiomers, and the total residue of spiroxamine (*via* 4-t-butylcyclohexanone)), the residues of trifloxystrobin (comprising trifloxystrobin, CGA 331409, CGA 357262, CGA 357261, CGA 321113 and CGA 373466) in/on wheat (green material) after one spray application with PTZ & SPX & TFS EC 280.3, an emulsifiable concentrate (EC) formulation containing 93.3 g/L prothioconazole, 107 g/L spiroxamine and 80 g/L trifloxystrobin.

Test site for the field phase E19RP088-01, E19RP088-02 was Bayer Crop Science BCSD, Elisabeth-Selbert-Strasse 4a, 40764 Langenfeld, Germany. The test site for the field phase E19RP088-03 was Bayer Crop Science SA-NV, J.E. Mommaertslaan 14, 1831 Diegem (Machelen), Belgium. The test site for the field phase E19RP088-04 was Bayer Crop Science SA-NV Netherlands, Energieweg 1, 3641 RT Mijdrecht, Netherlands.

The study measured residues immediately following a single application at the four trial locations (consisting of 0.140 kg a.s./ha prothioconazole, 0.161 kg a.s./ha spiroxamine and 0.120 kg a.s./ha trifloxystrobin).

The sample material to be analyzed was green material. The analysed substances were prothioconazole, JAU 6476-desthio, total residue of spiroxamine (*via* 4-t-butylcyclohexanone), KWG 4168-A1 enantiomer, KWG 4168-A2 enantiomer, KWG 4168-B1 enantiomer, KWG 4168-B2 enantiomer, total residue of 4 spiroxamine enantiomers, trifloxystrobin, CGA 321113, CGA 331409, CGA 357262, CGA 357261 and CGA 373466.

The application rates of the active substance(s) were calculated based on the nominal contents. No additional adjuvants, surfactants or mixing partners were used for the application.

Analytical method

Samples of wheat/green material were analysed using the validated analytical method 01480, report reference M-628347-02-1 (**see Doc MCP Section 5**).

II. Results and Discussion

Mean temperatures ranged from 7 to 14°C in the German trial (E19RP088-01), mean temperatures ranged from 12 to 20°C in the German trial (E19RP088-02), mean temperatures ranged from 9 to 12°C in the Belgium trial (E19RP088-03) and mean temperatures ranged from 8 to 17°C in the Netherlands trial (E19RP088-04).

Rainfall ranged from 0 to 8 mm in the German trial (E19RP088-01), 0 to 1 mm in the German trial (E19RP088-02), 0 to 6 mm in the Belgium trial (E19RP088-03) and 0 to 10 mm in the Netherlands trial (E19RP088-04).

The application rate of prothioconazole, spiroxamine and trifloxystrobin for each trial was 0.140, 0.161 and 0.120 kg a.s./ha, respectively.

Average recoveries were within the range of 70-110%. No residues above the LOQ were found in control samples with only two exceptions.

The residue levels determined in the treated samples are summarised in the tables below:

Table CP 10.1.1.2/34-1 Measured residues of prothioconazole and JAU 6476-desthio in/on wheat

Trial No. Country	BBCH growth stage	DALT	Residues (mg/kg)	
			Prothioconazole	JAU 6476-desthio
E19RP088-01 Germany	26	0	1.1	2.8
	26	1	0.16	2.9
	26	2	0.049	2.1
	30	3	0.037	1.9
	30	5	0.022	1.3
	30	7	0.014	0.84
	31	9	< 0.01	0.42
E19RP088-02 Germany	22	0	2.0	3.9
	22	1	0.12	3.8
	30	2	0.039	3.0
	30	3	0.041	2.7
	30	5	0.019	1.8
	31	7	0.019	1.0
	32	10	< 0.01	0.41
E19RP088-03 Belgium	23	0	2.3	5.4
	23	1	0.30	3.0
	23	2	0.10	2.3

Trial No. Country	BBCH growth stage	DALT	Residues (mg/kg)	
			Prothioconazole	JAU 6476-desthio
	23	3	0.042	1.6
	30	6	0.024	0.62
	30	7	0.019	0.47
	30	10	< 0.01	0.16
E19RP088-04 The Netherlands	29	0	3.1	2.1
	29	1	0.33	2.9
	30	2	0.048	1.8
	30	3	0.037	1.3
	31	5	0.024	1.1
	32	7	0.018	0.81
	32	10	0.014	0.52

DALT = Days after last application

Table CP 10.1.1.2/34-2 Measured residues of KWG 4168-A1 enantiomer, KWG 4168-A2 enantiomer, KWG 4168-B1 enantiomer, KWG 4168-B2 enantiomer and total residue of 4 spiroxamine enantiomer in/on wheat

Trial No. Country	BBCH growth stage	DALT	Residues (mg/kg)				
			KWG 4168-A1 enantiomer	KWG 4168-A2 enantiomer	KWG 4168-B1 enantiomer	KWG 4168-B2 enantiomer	total residue of 4 spiroxamine enantiomer
E19RP088-01 Germany	26	0	1.1	1.1	0.92	0.94	4.0
	26	1	0.71	0.71	0.61	0.62	2.6
	26	2	0.75	0.76	0.64	0.66	2.8
	30	3	0.59	0.60	0.51	0.53	2.2
	30	5	0.44	0.45	0.38	0.37	1.7
	30	7	0.37	0.37	0.32	0.31	1.4
	31	9	0.21	0.21	0.17	0.17	0.75
E19RP088-02 Germany	22	0	1.5	1.4	1.2	1.2	5.3
	22	1	0.91	0.91	0.74	0.75	3.3
	30	2	0.67	0.67	0.55	0.57	2.5
	30	3	0.63	0.63	0.51	0.53	2.3
	30	5	0.45	0.45	0.38	0.40	1.7
	31	7	0.34	.34	0.29	0.29	1.2
	32	10	0.24	0.24	0.20	0.20	0.88
	23	0	1.8	1.8	1.5	1.5	6.6

Trial No. Country	BBCH growth stage	DALT	Residues (mg/kg)				
			KWG 4168-A1 enantiomer	KWG 4168-A2 enantiomer	KWG 4168-B1 enantiomer	KWG 4168-B2 enantiomer	total residue of spiroxamine enantiomer
E19RP088-03 Belgium	23	1	1.1	1.1	0.98	1.0	4.2
	23	2	0.92	0.90	0.78	0.80	3.4
	23	3	0.75	0.75	0.65	0.63	2.8
	30	6	0.31	0.31	0.28	0.27	1.2
	30	7	0.27	0.27	0.22	0.23	0.98
	30	10	0.10	0.10	0.085	0.088	0.38
E19RP088-04 The Netherlands	29	0	1.7	1.7	1.4	1.4	6.1
	29	1	0.94	0.91	0.77	0.78	3.4
	30	2	0.37	0.36	0.30	0.30	1.3
	30	3	0.24	0.25	0.19	0.20	0.88
	31	5	0.18	0.18	0.14	0.14	0.65
	32	7	0.13	0.13	0.10	0.099	0.46
	32	10	0.10	0.096	0.073	0.074	0.34

DALT = Days after last application

Table CP 10.1.1.2/34-3 Measured residues of spiroxamine 1 (*via* 4-t-butylyclohexanone) in/on wheat

Trial No. Country	BBCH growth stage	DALT	Total residue of spiroxamine 1 (via 4-t-butylyclohexanone (mg/kg)
E19RP088-01 Germany	26	0	7.1
	26	1	4.7
	26	2	5.4
	30	3	4.3
	30	5	3.2
	30	7	2.4
	31	9	1.4
E19RP088-02 Germany	22	0	8.5
	22	1	6.5
	30	2	4.5
	30	3	5.1
	30	5	3.0
	31	7	2.3
	32	10	1.7

Trial No. Country	BBCH growth stage	DALT	Total residue of spiroxamine 1 (via 4-t-butylcyclohexanone (mg/kg)
E19RP088-03 Belgium	23	0	15
	23	1	6.7
	23	2	4.9
	23	3	3.8
	30	6	1.7
	30	7	1.5
	30	10	0.60
E19RP088-04 The Netherlands	29	0	8.3
	29	1	6.3
	30	2	2.4
	30	3	2.0
	31	5	1.4
	32	7	1.0
	32	10	0.83

DALT = Days after last application

Table CP 10.1.1.2/3.4.4 Measured residues of trifloxystrobin, CGA 321113, CGA 331409, CGA 357262, CGA 357261, CGA 373466 in/on wheat

Trial No. Country	BBCH growth stage	DALT	Residues (mg/kg)					
			Trifloxy-strobin	CGA 321113	CGA 331409	CGA 357262	CGA 357261	CGA 373466
E19RP088-01 Germany	26	0	5.2	0.42	0.056	< 0.01	0.12	0.013
	26	1	2.9	0.40	0.25	0.14	0.71	0.10
	26	2	1.9	0.18	0.30	0.31	0.98	0.11
	30	3	1.1	0.092	0.26	0.31	0.77	0.076
	30	5	0.55	0.057	0.18	0.23	0.45	0.050
	30	7	0.26	0.046	0.11	0.17	0.24	0.039
	31	9	0.067	0.013	0.049	0.064	0.047	< 0.01
E19RP088-02 Germany	22	0	6.8	0.30	0.12	0.016	0.22	0.016
	22	1	4.3	0.41	0.39	0.16	0.79	0.072
	30	2	2.9	0.28	0.47	0.27	0.93	0.068
	30	3	2.3*	0.18*	0.44*	0.28*	0.85*	0.054*
	30	5	0.96	0.11	0.33	0.22	0.34	0.029
	31	7	0.51	0.023	0.20	0.18	0.29	0.012
	32	10	0.25	0.016	0.15	0.13	0.15	< 0.01

Trial No. Country	BBCH growth stage	DALT	Residues (mg/kg)					
			Trifloxy-strobin	CGA 321113	CGA 331409	CGA 357262	CGA 357261	CGA 373466
E19RP088-03 Belgium	23	0	9.1*	0.45*	0.73*	0.28*	1.8*	0.10*
	23	1	3.5	0.32	0.48	0.19	0.59	0.076
	23	2	1.1	0.077	0.29	0.13	0.28	0.013
	23	3	0.48*	0.037*	0.20*	0.12*	0.20*	< 0.01*
	30	6	0.10	< 0.01	0.069	0.055	0.060	0.01
	30	7	0.080	< 0.01	0.053	0.047	0.053	< 0.01
	30	10	0.034	< 0.01	0.022	0.021	0.023	< 0.01
E19RP088-04 The Netherlands	29	0	10	0.16	0.12	0.065	0.25	0.01
	29	1	6.8	0.23	0.36	0.18	0.88	0.048
	30	2	2.8	0.13	0.31	0.16	0.34	0.017
	30	3	2.0	0.068	0.25	0.17	0.30	0.011
	31	5	1.2	0.036	0.21	0.16	0.24	< 0.01
	32	7	0.95	0.025	0.16	0.14	0.18	< 0.01
	32	10	0.94	0.022	0.12	0.11	0.16	< 0.01

DALT = Days after last application

*mean value, sample was extracted and analysed multiple times

No deviations occurred during the conduct of this study which had any negative impact on the quality of this study.

III. Conclusion

Residues of prothioconazole comprised of prothioconazole and its metabolite JAU 6476-desthio. Residues of spiroxamine comprised of the spiroxamine enantiomers A1, A2, B1 and B2, the total residue of spiroxamine parent as the sum of the four enantiomers, and the total residue of spiroxamine (*via* 4-tbutylcyclohexamone. Residues of trifloxystrobin comprising trifloxystrobin and its isomers/metabolites CGA 331409, CGA 357262, CGA 357261, CGA 321113 and CGA 373466.

Average recoveries were within the range of 70-110%. No residues above the LOQ were found in control samples with only two exceptions.

Assessment and conclusion by applicant:

There is no formal test guideline for the conduct of residues decline trials but some guidance is provided in the EFSA Guidance document on Risk Assessment for Birds and Mammals (2009) as well as the more recent EFSA Technical Report on general recurring issues in ecotoxicology (EFSA supporting publication 2019:EN-1673). Up to date regulatory intelligence on the expectations of the test design of such trials has also been taken in to consideration.

The study comprised four trials over three countries in NEU (Germany, the Netherlands and Belgium). The crop used was wheat which is highly relevant to the proposed representative GAP for cereals. The application rate was high enough in order to achieve sufficiently high residues of spiroxamine at the start of the trials, thereby allowing for a good decline curve to derive DT_{50} values from.

The analytical sampling regime adopted in the trials is considered to be suitable to derive reliable DT_{50} values, with sampling timepoints typically conducted on Days 0, 1, 2, 3, 5, 7 and 10. This regime is considered to meet the expectations for a robust residues decline trial, even with the relatively short expected DT_{50} for spiroxamine.

Weather data were adequately recorded from the nearest weather station. Rainfall was measured at the trial sites themselves in order to provide accurate precipitation measurements.

Overall, the data are considered reliable and suitable for inclusion in the derivation of refined DT_{50} values in cereals for use in Bird & Mammal risk assessment.

Report M-759383-01-1 presents the results of the kinetic modeling for the spiroxamine data measured for these trials as well as the other available decline studies on cereals. An overall DT_{50} value for spiroxamine has been determined using all of these data. As part of the analysis, trials that measured >1 mm rainfall in the first 24 hours were both included and excluded in order to assess the impact that this rainfall may have had on the overall DT_{50} value. It was concluded that there was very little variation in the mean DT_{50} value when these trials were either included or excluded. Thus, it is considered that any rainfall recorded in these trials has not adversely affected the results achieved therefore these trials are considered to be valid and can be included in the determination of the residue decline for spiroxamine.

Ecological data

The following ecological data are available and considered relevant to the proposed use of Prothioconazole + Spiroxamine EC 460 in cereals.

Data Point:	KCP 10.1.1.2/03
Report Author:	[redacted]
Report Year:	2006
Report Title:	Bird species in cereal fields in Germany, Poland, France and Italy: field data for the determination of focal species
Report No:	RA05-007
Document No:	M-299916-01-1
Guideline(s) followed in study:	EC Council Directive 91/414/EEC amended by the Commission Directive 96/68/EC; SANCO 4145/2000
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and accepted RAR (2010)
GLP/Officially recognised testing facilities:	No, not conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The objectives of this generic study were to determine the qualitative and quantitative composition of the bird community employing the parameters, frequency of occurrence (FO_{field} and FO_{survey}) and dominance both as overall and as cereal growth stage specific descriptors, respectively. Another objective was to then allocate the selected species to defined foraging guilds, diet guilds and size classes.

The results of the study are summarised as a list of candidates for focal bird species.

Study Area

Different regions of Germany, Poland, France and Italy served as study areas and encompassed 25, 24, 21 and 20 cereal fields, respectively (Germany: average transect length 599 ± 34 m, range 320 – 902 m, median 564 m, n = 25; Poland: average transect length 719 ± 41 m, range 357 – 1,002 m, median 695

m, n = 24; France: average transect length 728 ± 33 m, range 449 – 1,000 m, median 716 m, n = 21; Italy: average transect length 827 ± 36 m, range 557 –1,000 m, median 803 m, n = 20). The selected fields represent average cereal fields, cereal field size and the structure of the landscape.

I. Study design

The test system consisted of 25, 24, 21 and 20 commercial cereal fields with standard husbandry according to Good Agricultural Practice (GAP) in Germany, Poland, France and Italy, respectively.

Field observations covered a period of 6½ months in 2005, from late January to the beginning of July, including the following cereal growth stages. The assignment of growth stages to fields is not absolute since a variety of growth stages may be present on one and the same field at the same time. The average condition of the majority of crop plants was used to assign growth stages. Survey periods focussed on growth stages and consequently the individual surveys were conducted at slightly different times in the individual regions due to differences in the cultivation schedule.

The study was conducted at eight regions located in Germany, Poland, France and Italy. The study sites are situated in typical agricultural regions for cereal. A total of 89 cereal fields were examined by line transect counts. The number of line transects is equivalent to the number of cereal fields/study plots.

Most cereal fields were situated in open landscapes dominated by agricultural management and thus were surrounded mainly by other cereal fields interspersed with hedgerows, small woodlands and set-aside fields.

Cereal fields were visited between the end of January and beginning of July 2005. Three surveys were conducted in each field within a six month period. A standardised cereal field form and a bird survey form were developed to record a number of parameters during each field survey.

The avifauna of the cereal fields was surveyed by the line transect method for which general information can be found in Bibby *et al.* (1992). To meet the specific methodological requirements of this report, the line transect method described by Bibby *et al.* (1992) was adapted for this study, as described below.

All bird species were recorded in each cereal field by walking slowly along a defined longitudinal line transect, allowing for a clear view across the field. The length of the line transects was defined by the length of the cereal field. Each of the individual birds visually or acoustically registered was assigned to one of the following areas.

Only birds present (foraging, roosting, singing) in the 'in-crop transect band' of each cereal field were included for data analysis. Birds flying up to a height of 5 m above average crop height (*e.g.* actively hunting swallows, swifts or raptors) were also included in the analysis. Birds not directly associated with the cereal field, *e.g.* flying above 5 m over average crop height were assigned to the 'outside transect band' and ignored for the purposes of this analysis.

Frequency of occurrence

The frequency of occurrence (FO) can be determined in two different ways, related to the number of fields and related to the total number of surveys.

FO_{field} denotes the number of fields in which a defined species was recorded, given as percentage of the total number of fields regardless of the number of individuals observed. This approach serves as a measure for the spatial frequency of occurrence.

FO_{survey} denotes the number of surveys in which a defined species was recorded, given as percentage of the total number of surveys. This approach gives an approximation for the temporal evenness of occurrence throughout the complete study period.

Dominance

The dominance denotes the relative occurrence of bird species within the bird community. It is reported as the percentage number of individuals of the respective species compared to the total number of individuals throughout all species (calculated as arithmetic means over all cereal fields). Number of

individuals of a given bird species in all 21 cereal fields analysed. A species was termed 'dominant' when the dominance value of the species was greater than or equal to 5%.

Flocking behaviour

Dominance data may be biased by species with flocking behaviour. A method of aggregation was performed to separate between flocking and non-flocking species across and during different survey periods. To obtain this information, the number of individual bird contacts was plotted against the number of surveys, in which the respective species had been detected. To categorise flocking species and/or species with numerous occurrences a threshold value of on average more than five individual bird contacts per survey was chosen. For bird species occurring only in small groups values between on average 3 – 5 contacts per survey were chosen, whereas non-flocking species (rare or uniformly distributed species) were defined by values of on average less than three contacts per survey.

II. Results and Discussion

Germany

During the course of the study, a total of 562 individual bird contacts, comprising 20 different bird species, was recorded within the 'in-crop transect band' of the 25 cereal fields in Germany.

The highest frequency of occurrence across study plots (FO_{field}) was exhibited by the skylark (96.0%), followed by the tree sparrow (32.0%), white wagtail (28.0%), carrion crow (20.0%) and yellow wagtail (20.0%).

The highest time-weighted occurrence throughout the study period, as indicated by the FO_{survey} was recorded for the skylark (84.0%), followed by the tree sparrow (13.3%).

Table CP 10.1.1.2/03-1 Frequency of occurrence of bird species in relation to the total number of study plots in cereal fields in Germany

Species	FO_{field} [%]	FO_{survey} [%]	Dominance [%]
Skylark (*Alauda arvensis*)	96.0	84.0	47.5
Tree sparrow (*Passer montanus*)	32.0	13.3	3.6
White wagtail (*Motacilla alba*)	28.0	9.3	1.4
Yellow wagtail (*Motacilla flava*)	20.0	8.0	2.8
Carrion crow (*Corvus corone*)	20.0	6.7	1.8
Pheasant (*Phasianus colchius*)	16.0	6.4	1.4
Yellowhammer (*Emberiza citrinella*)	12.0	4.0	0.5
Grey partridge (*Perdix perdix*)	8.0	4.0	1.1
Linnet (*Carduelis cannabina*)	8.0	2.7	1.2
Starling (*Sturnus vulgaris*)	8.0	2.7	1.1
Golden plover (*Pluvialis apricaria*)	4.0	1.3	21.4
Lapwing (*Vanellus vanellus*)	4.0	1.3	14.2
House sparrow (*Passer domesticus*)	4.0	1.3	0.5
Mallard (*Anas platyrhynchos*)	4.0	1.3	0.4
Blackbird (*Turdus merula*)	4.0	1.3	0.2
Blackcap (*Sylvia atricapilla*)	4.0	1.3	0.2
Chiffchaff (*Phylloscopus collybita*)	4.0	1.3	0.2
Common buzzard (*Buteo buteo*)	4.0	1.3	0.2
Kestrel (*Falco tinnunculus*)	4.0	1.3	0.2
Whitethroat (*Sylvia communis*)	4.0	1.3	0.2

List of candidates of focal bird species in Germany

Four species were found to be characterised by an FO_{field} of at least 20%. Most of these species were grouped into the small bird category (< 50 g) and only one was large (carrion crow).

Table CP 10.1.1.2/03-2 List of candidates of focal bird species in Germany

Species	FO_{field} [a) b)] [%]	FO_{survey} [a) c)] [%]	Dominance [a) d)] [%]	Body weight [e)] [g]	Stratum use [f)]	Diet guild [g)]
Skylark (*Alauda arvensis*)	96.0	84.0	47.5	37.2	Ground	Omnivorous
Tree sparrow (*Passer montanus*)	32.0	13.3	3.6	22.0	Ground/ foliage	Omnivorous
White wagtail (*Motacilla alba*)	28.0	9.3	1.4	21.0	Ground	Insectivorous

Species	FO_{field} a) b) [%]	FO_{survey} a) c) [%]	Dominance a) d) [%]	Body weight e) [g]	Stratum use f)	Diet guild g)
Yellow wagtail (*Motacilla flava*)	20.0	8.0	2.8	17.6	Ground	Insectivorous
Carrion crow (*Corvus corone*)	20.0	6.7	1.8	570.0	Ground	Omnivorous

a) Across the complete study period (3 survey periods)

b) Based on 25 study plots (cereal fields)

c) Based on 75 surveys

d) Based on the individuals per study plot of one species in relation to the mean number of individuals per study plot of all species for a total number of 25 cereal fields

e) According to Dunning (1993). In case sex-specific values, the lower number was chosen

f) Predominant foraging stratum during growing season according to Perrins (1998)

g) Predominant diet composition during growing season according to Perrins (1998)

Poland

During the course of the study, a total of 552 individual bird contacts, comprising 30 different bird species, was recorded within the 'in-crop transect band' of the 24 cereal fields in Poland.

The frequency of occurrence of bird species in cereal fields in Poland is presented below. The highest frequency of occurrence (FO_{field}) was exhibited by the skylark (95.8%), followed by the yellow wagtail (66.7%) and corn bunting (37.5%).

The highest time-weighted occurrence throughout the study period, as indicated by the FO_{survey} was recorded for the skylark (94.4%), followed by the yellow wagtail (41.7%), corn bunting (20.8%) and barn swallow (11.1%).

Table CP 10.1.1.2/03-3 Frequency of occurrence of bird species in relation to the total number of surveys in cereal fields in Poland

Species	FO_{field} [%]	FO_{survey} [%]	Dominance [%]
Skylark (*Alauda arvensis*)	95.8	94.4	56.5
Yellow wagtail (*Motacilla flava*)	66.7	41.7	10.5
Corn bunting (*Miliaria calandra*)	37.5	20.8	3.8
Barn swallow (*Hirundo rustica*)	16.7	11.1	2.1
Montagu's harrier (*Circus pygargus*)	16.7	6.9	0.9
Starling (*Sturnus vulgaris*)	12.5	5.6	2.0
Whinchat (*Saxocila ruberta*)	12.5	5.6	1.3
Quail (*Coturnix coturnix*)	12.5	4.2	1.1
Grey partridge (*Perdix perdix*)	12.5	4.2	0.9
Marsh harrier (*Circus aeruginosus*)	8.3	4.2	0.5
Wood pigeon (*Columba palumbus*)	8.3	4.2	0.7
White stork (*Ciconia ciconia*)	8.3	4.2	0.5
Yellowhammer (*Emberiza citrinella*)	8.3	4.2	0.5
House sparrow (*Passer domesticus*)	8.3	2.8	4.0
Lapwing (*Vanellus vanellus*)	8.3	2.8	0.4
Ortolan bunting (*Emberiza hortulana*)	4.2	2.8	0.4
Linnet (*Carduelis cannabina*)	4.2	2.8	0.5
Fieldfare (*Turdus pilaris*)	4.2	1.4	7.6
Song thrush (*Turdus philomelos*)	4.2	1.4	1.1
Carrion crow (*Corvus corone*)	4.2	1.4	0.7
Tree sparrow (*Passer montanus*)	4.2	1.4	0.5
Jackdaw (*Corvus corone*)	4.2	1.4	0.4
Magpie (*Pica pica*)	4.2	1.4	0.4
Redwing (*Turdus iliacus*)	4.2	1.4	0.4
Common buzzard (*Ruteo buteo*)	4.2	1.4	0.2
Great grey shrike (*Lanius excubitor*)	4.2	1.4	0.2
Meadow pipet (*Anthus pratensis*)	4.2	1.4	0.2
Pheasant (*Phasianus colchius*)	4.2	1.4	0.2
Raven (*Corvus corax*)	4.2	1.4	0.2
White wagtail (*Motacilla alba*)	4.2	1.4	0.2

List of candidates of focal bird species in Poland

Three species were found to be characterised by an FO_{field} of at least 20%. All these species were grouped into the small bird category (< 50 g).

Table CP 10.1.1.2/03-4 List of candidates of focal bird species in Poland

Species	FO_{field} a) b) [%]	FO_{survey} a) c) [%]	Dominance a) d) [%]	Body weight [g]	Stratum use f)	Diet guild g)
Skylark (*Alauda arvensis*)	95.8	94.4	56.5	37.2	Ground	Omnivorous
Yellow wagtail (*Motacilla flava*)	66.7	41.7	10.5	17.6	Ground	Insectivorous
Corn bunting (*Miliaria calndra*)	37.5	20.8	6.8	46.0	Ground	Omnivorous

a) Across the complete study period (3 survey periods)

b) Based on 24 study plots (cereal fields)

c) Based on 75 surveys2

d) Based on the individuals per study plot of one species in relation to the mean number of individuals per study plot of all species for a total number of 24 cereal fields

e) According to Dunning (1993). In case sex-specific values, the lower number was chosen

f) Predominant foraging stratum during growing season according to Perrins (1998)

g) Predominant diet composition during growing season according to Perrins (1998)

France

During the course of the study, a total of 703 individual bird contacts, comprising 10 different bird species, was recorded within the 'in-crop transect band' of the 21 cereal fields in France.

The highest frequency of occurrence (FO_{field}) was exhibited by the yellow wagtail (85.7%), followed by the skylark (81.0%), quail (52.4%), corn bunting (38.1%) and grey partridge (23.8%).

The highest time-weighted occurrence throughout the study period, as indicated by the FO_{survey} was recorded for the skylark (55.6%), followed by the yellow wagtail (42.9%), quail (17.5%) and corn bunting (15.9%).

Table CP 10.1.1.2/03-5 Frequency of occurrence of bird species in relation to the total number of surveys in cereal fields in France

Species	FO_{field} [%]	FO_{survey} [%]	Dominance [%]
Yellow wagtail (*Motacilla flava*)	85.7	42.9	8.7
Skylark (*Alauda arvensis*)	81.0	55.6	38.4
Quail (*Coturnix coturnix*)	52.4	17.5	2.0
Corn bunting (*Miliaria calandra*)	38.1	15.9	2.0
Grey partridge (*Perdix perdix*)	23.8	7.9	2.1
Hen harrier (*Circus cyaneus*)	19.0	7.9	1.5
Carrion crow (*Corvus corone*)	14.3	4.8	1.0
Crane (*Grus grus*)	9.5	3.2	4.1
Linnet (*Carduelis cannabina*)	4.8	1.6	0.3
Common buzzard (*Buteo buteo*)	4.8	1.6	0.1

List of candidates of focal bird species in France

Five species were found to be characterised by an FO_{field} of at least 20%. Three of these species were grouped into the small bird category (< 50 g) and two were medium-sized (50 – 500 g).

Table CP 10.1.1.2/03-6 List of candidates of focal bird species in France

Species	FO_{field} [a) b)] [%]	FO_{survey} [a) c)] [%]	Dominance [a) d)] [%]	Body weight [e)] [g]	Stratum use [f)]	Diet guild [g)]
Yellow wagtail (*Motacilla flava*)	85.7	42.9	8.7	17.6	Ground	Insectivorous
Skylark (*Alauda arvensis*)	81.0	55.6	38.4	37.2	Ground	Omnivorous
Quail (*Coturnix coturnix*)	52.4	17.5	2.0	90.0	Ground	Omnivorous
Corn bunting (*Miliaria calndra*)	38.1	15.9	2.0	46.0	Ground	Omnivorous
Grey partridge (*Perdix perdix*)	23.8	7.9	2.1	381.0	Ground	Omnivorous

a) Across the complete study period (3 survey periods)

b) Based on 21 study plots (cereal fields)

c) Based on 63 surveys2

d) Based on the individuals per study plot of one species in relation to the mean number of individuals per study plot of all species for a total number of 21 cereal fields

e) According to Dunning (1993). In case sex-specific values, the lower number was chosen

f) Predominant foraging stratum during growing season according to Perrins (1998)

g) Predominant diet composition during growing season according to Perrins (1998)

Italy

During the course of the study, a total of 996 individual bird contacts, comprising 30 different bird species, was recorded within the 'in-crop transect band' of the 20 cereal fields in Italy.

The highest frequency of occurrence across study plots (FO_{field}) was exhibited by the crested lark (100.0%), followed by the corn bunting and skylark (95.0% each), short-toed lark (60.0%), stonechat (45.0%), quail (40.0%), house sparrow, linnet, meadow pipit, red-footed falcon, whinchat (35.0% each), magpie (30.0%), fan-tailed warbler and yellow wagtail (25.0% each).

The highest time-weighted occurrence throughout the study period, as indicated by the FO_{survey}, was recorded for the skylark (81.7%), followed by the crested lark (80.0%), corn bunting (56.7%), short-toed lark (20.0%), house sparrow (18.3%), stonechat (16.7%), meadow pipit and quail (13.3% each).

Table CP 10.1.1.2/03-7 Frequency of occurrence of bird species in relation to the total number of surveys in cereal fields in Italy

Species	FO_{field} [%]	FO_{survey} [%]	Dominance [%]
Crested lark (*Galerida cristata*)	100.0	80.0	24.9
Skylark (*Alauda arvensis*)	95.0	81.7	28.4
Corn bunting (*Miliaria calandra*)	95.0	56.7	12.9
Short-toed lark (*Calandrella brachydactyla*)	60.0	20.0	3.7
Stonechat (*Saxicola rubicola*)	45.0	16.7	2.3
Quail (*Coturnix coturnix*)	40.0	13.3	0.9
House sparrow (*Passer domesticus*)	35.0	18.3	6.2
Meadow pipet (*Anthus pratensis*)	35.0	13.3	3.5
Linnet (*Carduelis cannabina*)	35.0	11.7	3
Red-footed falcon (*Falco vespertinus*)	35.0	11.7	2.8
Whinchat (*Saxocila ruberta*)	35.0	11.7	1.2
Magpie (*Pica pica*)	30.0	11.7	0.7
Fan-tailed warbler (*Cisticola juncidis*)	25.0	8.3	0.6
Yellow wagtail (*Motacilla flava*)	25.0	8.3	0.6
Goldfinch (*Carduelis carduelis*)	15.0	5.0	1.6
Hoopoe (*Upupa epops*)	15.0	5.0	0.4
Calandra lark (*Melanocorypha calandra*)	10.0	3.0	1.3
Black redstart (*Phoenicurus ochruros*)	10.0	3.3	0.3
Kestrel (*Falco tinnunculus*)	10.0	3.3	0.2
Marsh harrier (*Circus aeruginosus*)	10.0	3.3	0.2
Serin (*Serinus serinus*)	10.0	3.3	0.2
White wagtail (*Motacilla alba*)	5.0	1.7	0.5
Tree sparrow (*Passer montanus*)	5.0	1.7	0.4
Spanish sparrow (*Passer montanus*)	5.0	1.7	0.2
Blackcap (*Sylvia atricapilla*)	5.0	1.7	0.1
Feral pigeon (*Columba livia f. domestica*)	5.0	1.7	0.1
Great reed warbler (*Acrocephalus arundinaceus*)	5.0	1.7	0.1
Hen harrier (*Circus cyaneus*)	5.0	1.7	0.1
Hooded crow (*Corvus cornix*)	5.0	1.7	0.1
Montagu's harrier (*Circus pygargus*)	5.0	1.7	0.1

List of candidates of focal bird species in Italy

Fourteen species were found to be characterized by an FO_{field} of at least 20%. Most of these species were grouped into the small bird category (< 50 g) and only three were medium-sized (quail, red-footed falcon, magpie).

Table CP 10.1.1.2/03-8 List of candidates of focal species in cereal fields in Italy

Species	FO_{field} a) b) [%]	FO_{survey} a) c) [%]	Dominance a) d) [%]	Body weight e) [g]	Stratum use f)	Diet guild e)
Crested lark (*Galerida cristata*)	100.0	80.0	24.9	39.0	Ground	Omnivorous
Skylark (*Alauda arvensis*)	95.0	81.7	28.4	37.2	Ground	Omnivorous
Corn bunting (*Miliaria calandra*)	95.0	56.7	12.9	46.0	Ground	Omnivorous
Short-toed lark (*Calandrella brachydactyla*)	60.0	20.0	3.7	22.0	Ground	Omnivorous
Stonechat (*Saxicola rubicola*)	45.0	16.7	2.3	16.3	Ground	Insectivorous
Quail (*Coturnix coturnix*)	40.0	13.3	0.9	90.0	Ground	Omnivorous
House sparrow (*Passer domesticus*)	35.0	18.3	18.3	27.4	Ground/ foliage	Omnivorous
Meadow pipet (*Anthus pratensis*)	35.0	13.3	3.5	18.4	Ground	Insectivorous
Linnet (*Carduelis cannabina*)	35.0	13.7	5.3	15.3	Ground	Granivorous
Red-footed falcon (*Falco vespertinus*)	35.0	11.7	2.8	2.8	Ground	Insectivorous
Whinchat (*Saxicila ruberta*)	35.0	11.7	1.2	1.2	Ground/ foliage	Insectivorous
Magpie (*Pica pica*)	30.0	11.7	0.7	0.7	Ground/ foliage	Omnivorous
Fan-tailed warbler (*Cisticola juncidis*)	25.0	8.3	0.6	0.6	Foliage	Insectivorous
Yellow wagtail (*Motacilla flava*)	25.0	8.3	0.6	0.6	Ground	Insectivorous

a) Across the complete study period (3 survey periods)

b) Based on 21 study plots (cereal fields)

c) Based on 63 surveys2

d) Based on the individuals per study plot of one species in relation to the mean number of individuals per study plot of all species for a total number of 21 cereal fields

e) According to Dunning (1993). In case sex-specific values, the lower number was chosen

f) Predominant foraging stratum during growing season according to Perrins (1998)

g) Predominant diet composition during growing season according to Perrins (1998)

III. Conclusion

In this generic study the frequency of occurrence was used to determine a list of candidates of focal bird species in cereal fields in Germany, Poland, France and Italy that could be used for assessing the risk of plant protection products to wild bird species in a refined assessment.

The frequency of occurrence (FO_{field}; FO_{survey}) and dominance were considered to be the decisive parameters for the derivation of focal species at a given period of time. The derivation of these parameters was based on both visual and acoustic identification of bird species. Due to this form of appraisal, there is potential for recording bias towards conspicuous species (loud song, *e.g.* skylark), which might be recorded more frequently compared to more elusive species (inconspicuous song/calls, *e.g.* linnet), which might be under-represented. Indeed, the detectability of a species decreases with increasing distance from the observer – particularly for inconspicuous species – and for this reason, the data analysis is restricted to birds observed and heard within the 100 m 'in-crop transect band' (50 m to each side of the observer) where this bias is negligible (Bibby *et al.* 1992).

As noted earlier (see 8.6), the cut-off criterion for FO_{field} ≥ 20% was defined arbitrarily.

However, the field data show that bird species reaching this cut-off criterion were the most abundant bird species.

The main criterion for selecting candidate focal species was the FO_{field} value. But also the ranking of species based on FO_{survey} showed almost no differences compared to FO_{field} values.

The FO_{field} value describes the likelihood of a defined species to occur on any particular cereal field, *i.e.* species with high FO_{field} values are likely to be present in a large number of fields of the crop over the entire season.

The FO_{survey} is more indicative of time-weighted occurrence (opposed to spatial as described by FO_{field}). This value is usually also high for species with high FO_{field} values, but differences caused by seasonal aspects are not detectable here. For example, a low FO_{survey} may be based on the occurrence of a species restricted to one crop stage only but over a large number of cereal fields (high FO_{field}), as in the case of migratory species. On the other hand, a species may occur throughout the season but only on a limited number of fields, resulting in a low FO_{survey} and a low FO_{field} value.

The dominance value requires careful interpretation since it may be biased by the flocking behaviour of some species. Species that exhibit only temporary flocking behaviour achieve high dominance values while the FO is rather low. To shed some light on this aspect the number of surveys in which a species was recorded is plotted against the number of recorded individuals of the same species for each survey period. Three categories were distinguished: 1) species occurring with on average less than three individuals per survey (scarce species), 2) species occurring with on average 3 – 5 individuals per survey (small groups, family party) or 3) species occurring with on average more than five individuals per survey (flocking species). Particularly these latter species are higher ranked according to dominance values than with respect to frequency of occurrence (FO_{field}, FO_{survey}).

FO_{field} values were given more significance than FO_{survey} or dominance values. However, FO_{survey} values can indicate the importance of the crop for a particular species across several growth stages (time-weighted frequency of occurrence) and the dominance value will help to identify flocking species compared to rare species. Therefore, the selection of focal species as presented in this report is justified and provides a good overview of the bird species occurring regularly in cereal fields in Germany, Poland, France and Italy.

Focal species in cereal fields in Germany, Poland, France and Italy at least at a certain period are summarised in the table below.

Table CP 10.1.1.2/03-9 List of focal species in cereal fields in Germany, Poland, France and Italy

Guild	**Country**			
	Germany	**Poland**	**France**	**Italy**
Small granivore (<50 g)				
Combined stratum user	-	-	-	Linnet [9]
Small insectivore (<50 g)				
Combined stratum user	-	-	-	Whinchat [11]
Ground dweller	White wagtail [3] Yellow wagtail [4]	Yellow wagtail [2]	Yellow wagtail [1]	Stonechat [5] Meadow pipet [8] Yellow wagtail [14]
Foliage dweller	-	-	-	Fan-tailed warbler [13]
Small omnivore (<50 g)				
Combined stratum user	Tree sparrow [2]	-	-	House sparrow [7]
Ground dweller	Skylark [1]	Skylark [1] Corn bunting [3]	Skylark [2] Corn bunting [4]	Crested lark [1] Skylark [2] Corn bunting [3] Short-toed lark [4]
Medium insectivore (50 – 500 g)				
Ground dweller	-	-	-	Red-footed falcon [10]
Medium omnivore (50 – 500 g)				
Ground dweller	-	-	Quail [3] Grey partridge [5]	Quail [6]
Combined stratum user	-	-	-	Magpie [12]
Large omnivore (>500 g)				
Ground dweller	Carrion crow [5]	-	-	-

Assessment and conclusion by applicant:

This ecological monitoring study was not conducted to GLP nor was it conducted according to a specific test guideline. However, this is typical of studies of this type therefore the study is still considered to be acceptable for regulatory purposes by supporting various aspects of the Bird & Mammal risk assessment.

The results of this study have been used as part of the refined risk assessment of the small omnivorous bird “lark” scenario, specifically to support the use of the skylark as a suitable focal species in cereal fields. Out of the sites studied, the skylark was found to be the most aboundant species present in cereal fields in Germany and Poland and in the top two most abundant species in France and Italy, thereby clearly demonstrating its suitability as a focal species for the refined risk assessment.

Data Point:	KCP 10.1.1.2/04
Report Author:	[redacted]
Report Year:	2007
Report Title:	Generic field monitoring of birds in cereal fields in spring and summer in Germany
Report No:	BAR/FS 034
Document No:	M-292641-01-1
Guideline(s) followed in study:	The test was specifically designed for this study
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and accepted RAR (2010)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

In this generic field study, a radio-tracking program was carried out around winter cereal fields in the region of '[redacted]' to assess which birds utilise cereal fields and their exposure to plant protection products. The focus was placed on autumn sown winter cereals during spring and summer.

Three main focal species were selected: Yellowhammer (*Emberiza citronella*), Tree Sparrow (*Passer montanus*) and Quail (*Coturnix coturnix*).

The proportion of time spent 'potentially foraging' was acquired by radio tracking and information on selected food items was gained from measurement of faecal and stomach content.

The study provided revised input data (PT and food choice) for the recalculation of toxicity to exposure ratios (TER) based on the risk of exposure due to foraging preferences.

Study area

The study was conducted in the region of '[redacted]', a typical area for winter cereal cultivation in Germany. The open landscape is dominated by large arable fields and is known to hold an essential population of three preselected focal species: Yellowhammer (*Emberiza citronella*), Tree Sparrow (*Passer montanus*) and Quail (*Coturnix coturnix*). The main crops were winter wheat, winter barley, oilseed rape, sugar beet and peas. Field sizes varied strongly, with the main crops being winter wheat, winter barley, oilseed rape, sugar beet and peas. Study fields ranged from 130 to 200 meters above sea level. All sites were commercially cultivated fields and treated according to Good Agriculture Practice (GAP). Predominant soil types of the study area were clay loam, loam and sandy loam.

I. Methods

The study was carried out between 21 March 2005 and 29th November 2005.

Crops and crop stages

Crop stages (BBCH codes) were surveyed approximately every two weeks during the course of the study. BBCH principal growth stages were classified into three groups:

BBCH principal growth stages	Comment

0-2	From germination to tillering
3-6	From stem elongation to flowering
7-9	All fruit stages

To evaluate the extent birds utilise freshly drilled winter cereals four different methods were applied in the course of the study: transect counts, scan sampling, radio tracking and food composition.

Transect counts

Prevalence and abundance of species within winter cereal fields and surrounding habitats were surveyed. Birds were counted and observed weekly on four defined transect walks within the area of the agrarian co-operative Warnstedt, all passing at least one winter cereal field. The behaviour of each individual was noted for the following categories: foraging, reproductive behaviour, resting, flying over, others (which were specified) or flushed by the observer. Dominance, abundance and prevalence were calculated for each passed habitat.

Dominance denotes the percentage of individuals of a certain species compared to the total number of individuals of all species observed. To estimate abundance, the cumulated bird numbers counted during all transect counts were divided by the cumulated area of each habitat type during all transect counts. The data can therefore be read as 'individuals per transect count per ha' or because the numbers are standardised for one transect, as 'individuals per ha'. Prevalence is illustrated by their frequency of occurrence per transect count (FO = percentage counts during which a given species was observed).

$$FO_{COUNT} = \frac{N_{POS} \times 100}{N_{TOTAL}}$$

For calculations, non-crop habitats were pooled to the category 'other'. This included development area, orchard, pine forest, fallow, dry grassland, path, hedge, scrub, vertical structures as well as the behaviour category 'flying over'.

Scan sampling

Six fields were observed from dawn to dusk on four days each. Every ten minutes a defined section of the study field was scanned with a binocular and spotting scope. Parallel but time-staggered to the observation of the cereal fields birds in adjacent off-crop habitats were registered in the same way every 30 minutes. For each scan the following parameters were recorded: species, number of individuals and their behaviour (foraging, active, inactive or other). Observations were made out of a car to minimise disturbance.

Analysis of the scan sampling data included calculation of species dominance (percentage of observed individuals), abundance (individuals per hectare) and prevalence as frequency of occurrence per scan and species (FO, percentage of scans with species present) in consideration of birds' behaviour. Dominance describes the percentage of species during each scan. Abundance was calculated by counting bird numbers from all scans divided by the cumulated area of all scan fields during all scans ('individuals per ha').

Frequency of occurrence was determined for each species and session separately *i.e.* for each of the 24 sessions the percentage of scans a given species was observed.

$$FO_{Session} = \frac{N_{POS} \times 100}{N_{TOTAL}}$$

The mean of the results for each species of all 24 sessions was calculated separately. As a worst-case assumption, the behavioural observation categorised 'active' and 'foraging' were summarised to 'potentially foraging'. To account for findings that a large number of birds staying on the field for a short time or a smaller number of birds remaining for a longer period show the same abundance, the

relative risk index (RR) is calculated. The index also accounts for the fact that the latter scenario is higher risk, since they are longer exposed to the treated food. The RR is defined as:

RR = n / nmin

The index varies from 0 to 1 (0: no risk; 1: high risk)

Radio telemetry of birds

Thirteen Yellowhammers, ten Quails and three Tree Sparrows were trapped and tagged with radio transmitters.

Birds were trapped using mist nets. To avoid adverse effects caused by tag-load, radio transmitters of less than 5% of the bird's body weight were used. Transmitters tended to fall off after some weeks.

The aim of the tracking was to monitor the foraging behaviour of individual birds as precisely as possible. Emphasis was placed on spatial and temporal feeding activities and habitats used by the birds, with special focus on the portion of time the focal species spent foraging in cereal fields.

Due to the birds' high mobility and their preferred sojourning in often dense vegetation, it was not always possible to accurately determine their position and behaviour. If the position could not be defined the category 'unknown' was assigned. Typically however, it was possible to distinguish whether the bird was inactive, active possibly foraging, active unknown, active known and reproductive behaviour. Foraging, active possibly foraging and unknown were summarised into one category called potentially foraging.

Yellowhammers and Tree Sparrows were continuously radio-tracked for one or two daylight periods. Since Quails are known to be active also during night each individual was tracked for at least one 24 hour session. To consider seasonal changes in the food choice of birds, potentially foraging behaviour was analysed according to BBCH principal growth stages.

Analysis of the birds' preference for feeding habitats was done using Jacobs' preference index [D]) which was calculated for each tracking session. Jacobs' index compares the utilisation of a habitat to its availability. It describes the relation between the proportion of time that a habitat was used for 'potentially foraging' and the home-range's habitat spatial proportion. The index (D) varies from -1 to 0 for negative selection and from 0 to +1 for positive selection (-1:complete avoidance; +1:string preference; 0:habitat neutral).

Diet sample composition

Samples of faeces or stomach contents were collected to gain information about the selected food items. To quantify diets sample composition, two different methods were used; proportion of volume and numerical proportion of food types. The proportion of volume of herbal and animal material was estimated during analysis. The number of invertebrates within the samples was calculated by estimating the minimum number of individuals required to account for the fragments of each prey type. In plant material, the number of fruits and seeds were obtained by measuring the area of the fragments and dividing this figure by the area of a reference fruit or seed. From remains of leaves or stems the area or length of the found particle was measured, recorded and supplemented by the value of estimated length of the ingested food item.

The numerical proportion of different food types ingested by the birds was calculated from their remains found in stomach flushing and faeces samples. Seeds were grouped in the categories 'cereal seeds' and 'other seeds'. All other vegetal food items were grouped in the category 'other plant material'. Arthropods, adult and larval, of the order Coleoptera, Dermaptera, Diptera and Hymenoptera, Lepidoptera and Rhynchota and Araneae were grouped separately. All other animal food items were identified and grouped in 'other animals'.

Additional observations

The daily average temperature data were obtained from the nearest climate recording station at Gemrode. Precipitation data were received from the 'Agrargenossenschaft Warnstedt e. G' in Warnstedt. The distance between Gernrode and Warnstedt was 7km.

II. Results and Discussion

The mean temperature during the study period was 4.4°C below zero, the maximum temperature was 32.5°C. Total precipitation was 271.7 mm, with a daily average of 1.69 mm per day.

Transect counts

Transect counts were performed on four different tracks, each walked 20 times, with a total area of 116.24 ha. In total 8365 individual sightings, of 82 different species were recorded. Species composition, frequency of occurrence and abundance differed considerably between crops and adjacent non-crop habitats.

Birds in winter cereal fields in spring and summer

Among the 82 species, 19 were found in winter cereal fields. Details of the species found on both spring and summer are presented below.

Table CP 10.1.1.2/04-1 Bird abundance in winter cereal field according to transect counts

Abundance of focal species in spring and summer			
Species in spring	Ind./transect count and ha	Species in summer	Ind./transect count and ha
Skylark	0.704	Skylark	0.566
Wood pigeon	0.029	House Sparrow	0.267
Yellowhammer	0.007	Tree Sparrow	0.126
Quail	-	Yellowhammer	0.023
Tree Sparrow	-	Quail	0.019
Blackbird	0.007	Blackbird	0.009

Birds in winter cereal fields of different growth stages

Plant growth had an influence on bird abundance in winter cereal fields. During BBCH growth stages 0-2 only three bird species were observed: Skylark (0.322 ind./transect count and ha), Starling (0.129 ind./transect count and ha) and Yellowhammer (0.043 ind./transect count and ha). During BBCH growth stages 3-6 the abundance of Skylark doubled (0.727 ind./transect count and ha) and altogether twelve species were noted. During BBCH growth stages 7-9 a total of fourteen species were detected. Besides the Skylark (0.587 ind./transect count and ha), the House Sparrow (0.364 ind./transect count and ha) and the Tree Sparrow (0.160 ind./transect count and ha) reach abundances greater than 0.05 ind./transect count and ha. Overall, abundance of birds increased from 0.494 ind./transect count and ha (growth stages 0-2) over 0.866 ind./transect count and ha (growth stages 3-6) to 1.252 ind./transect count and ha (growth stages 7-9).

Birds in winter cereal fields compared to other habitats

Bird diversity was lower in winter cereal fields than in most of the other habitats. Overall 19 species were detected. Only in pasture, plain field and summer oat less species were recorded. The highest diversity was found non-crop habitats (34 to 49 species) and oilseed rape (41 species).

Abundance and habitat use of the focal species

The Yellowhammer was most abundant in non-crop habitats for both spring and summer (hedge/scrub. Pine forest, pasture, dry grassland and orchard). The mean abundance in arable crops was considerably lower.

Habitat use of the Tree Sparrow changed notably between spring and summer. Winter wheat and winter barley were only used in summer.

Due to the Quail's late arrival, it could only be recorded in summer. Mean abundances were highest in summer oat.

The transect count approach offered the possibility to compare abundance in different habitats, monitoring a great variety in a short time. However, the data only gives a brief insight into the bird community. The transect count approach is not considered adequate for measuring abundance of Quail due to their special biology. This includes the calling in evening, avoidance of field borders, and a tendency to aggregate and thus not being evenly distributed throughout a given habitat. Consequently, to obtain better data on Quail, transect lines should be longer than those used in the current study.

Scan sampling

The most abundant species counted on winter cereal field was the Fieldfare with an average 0.101 ind./scan and ha, followed by the Song Thrush with on average 0.066 ind./scan and ha. Yellowhammer was on average 0.006 ind./scan and ha and < 0.001 ind./scan and ha for the Tree Sparrow. Due to the late arrival of the Quail, no information was available for this species.

The behaviour of the majority of birds was classified as potentially foraging, with only 4.5% displaying other behaviours.

The highest mean frequency of occurrence per scan day showed the Skylark (15.72%), followed by the Song Thrush (9.52%), Blackbird (4.60%) and Mistle Thrush (4.43%). All other species were below 3%, with Yellowhammer at 2.83% and Tree Sparrow at 0.15%. The highest maximum frequency of occurrence showed the Song Thrush (51.81%), Skylark (35.5%), with the Yellowhammer and Tree Sparrow accounting for 16.67% and 2.56%, respectively.

Calculation of RR showed that none of the identified species was exposed to a relatively high risk. The highest index was calculated for the Skylark (RR=0.20). For both Yellowhammer and Tree Sparrow the RR was below 0.15.

Bird diversity was higher in non-crop habitats (51 species) than in winter cereal fields (28 species). The most abundant species in off-crop habitats was the starling (0.446 ind./scan and ha) followed by the Yellowhammer (0.208 ind./scan and ha). In contrast to winter cereal fields, the portion of potentially foraging individuals in non-crop habitats was much lower, with 37.3% showing other behaviour.

The scan sampling method was used to obtain additional information about bird activity in the early stage of winter cereal growth as radio tracking was delayed. The results have to be considered carefully. Plant growth increasingly limited bird observations and thus affected the comparability of the data over the four week period. Furthermore, bigger birds like Thrushes were easier to detect between the plants and therefore may be over-represented in the data.

Radio tracking and tracking data compilation

Tracking sessions were conducted with Yellowhammers (25 sessions), Quails (13 sessions) and Tree Sparrows (3 sessions).

Yellowhammer

Twelve birds were tracked twice and one bird was tracked once. Yellowhammers spent an average of 42.27% of their time 'potentially foraging'. Most of their 'potentially foraging' time was spent in 'tree/bush/hedges' (mean = 53.62%), followed by 'winter cereals' (mean = 23.01%) and 'oilseed rape' (mean = 11.79%). All birds except one were at least once detected as 'potentially foraging' in winter cereals. Yellowhammers used cereal field for 'potentially foraging' mostly in summer June and July, when the crops were ripe.

The results of the Jacobs' index do not indicate a preferences for the habitat winter cereal, with a mean negative index (D = -0.71). Only one bird showed a positive index for this habitat.

Quail

Seven birds were tracked once and three birds twice. Quail spent on average 58.35% of their time 'potentially foraging. The observed birds either used winter cereals (mean = 64.27%) or pea fields (mean = 35.55%) as foraging sites. Tracked Quails were observed to be 'potentially foraging' during two sessions in winter cereals of BBCH principal growth stage 3-6 and in five sessions in winter cereals of growth stages 7-9. No significant difference between growth stages 3-6 and 7-9 were found for 'potentially foraging time' in winter cereals.

The average Jacobs' index for winter cereals in Quails was positive, indicating a preference for winter cereals (D = 0.39).

Tree Sparrow

Three birds were tracked once. Tree Sparrows spent most of their time potentially foraging in oilseed rape (mean = 32.24%), tree/bush/hedges (mean = 26.66%) and/or winter cereals (mean = 21.71%). Street/path (mean – 16/13%) and grassland (mean = 3.13%) were also used to a lesser extent. Tracked Tree Sparrows were only observed in winter cereals of BBCH principal growth stages 3-6, therefore no seasonal influences could be investigated.

The Jacobs' index for Tree Sparrows was negative indicating a slight avoidance of winter cereals (D=-0.34).

Table CP 10.1.1.2/04-2 Overview of potential foraging time values in winter cereal fields

Proportion of Time 'potentially foraging' in winter cereal fields in focal species			
Species	Mean (%)	90th %ile (%)	Tracking sessions (individuals)
Yellowhammer	23.01	69.72	25 (13)
Quail	64.27	100.00	13 (10)
Tree Sparrow	21.71	44.99	3 (3)

Table CP 10.1.1.2/04-3 Habitat preference according to radio tracking (using Jacobs preference index (D))

Jacobs Index (D), range: -1 to +1			
Species	Mean (%)	90th %ile (%)	Tracking sessions (individuals)
Yellowhammer	-0.71	-0.16	23 (13)
Quail	0.39	1.00	13 (10)
Tree Sparrow	-0.34	0.12	3 (3)

The radio tracking approach offered very accurate information on the home ranges and time budgets of the individually tracked birds of all focal species, concerning habitats, behaviour and feeding sites. There was no impact on the birds behaviour observed by trapping, handling and tagging procedures.

Food composition

The main food source of the Yellowhammer according to estimated volume-proportion was arthropod material (69%), whereas herbal material accounted for 31%. Main food items according to numerical proportions were Coleoptera (28.2%).

Main food source of the Quail according to estimated volume-proportion was arthropod material (65.4%), with plant material accounting for 34.6%. According to numerical proportions, the major food item was Coleoptera (29.9%).

Main food source for the Tree Sparrow according to estimated volume-proportion was plant material (61.3%), whereas arthropod material accounted for 38.7%. The major food item ingested by Tree Sparrows according to numerical proportions were other seeds (50.2%).

Table CP 10.1.1.2/04-4 Diet composition of focal species in winter cereal fields

Food Item	Numerical proportion (%)		
	Yellowhammer (n = 15)	Quail (n = 14)	Tree Sparrow (n = 10)
Cereal seeds	16.0	0.7	2.7
Other seeds	5.2	14.9	50.2
Other plant	2.8	9.7	15.1
Aranae	3.8	2.2	1.4
Coleoptera	28.2	29.9	11.9
Coleoptera		0.7	0.5
Dermaptera	0.5	22.4	3.2
Diptera	12.7	9.7	3.7
Diptera larvae	0.5	0.7	0.9
Hymenoptera	5.6	0.7	3.7
Hymenoptera	1.4	0.7	-
Lepidoptera	0.5	1.5	-
Lepidoptera	5.6	3.0	3.7
Rhynchota	11.3	3.0	2.7
Other animals	6.1	-	0.5

III. Conclusion

Radio tracking of Yellowhammers, Quails and Tree Sparrows in an agrarian landscape with a high number of winter cereal fields (wheat and barley) in the western part of Sachsen-Anhalt showed that this field type represents a significant habitat especially for the Quail, but to a lesser extent also for Tree Sparrow and Yellowhammer.

Radio tracking of thirteen individual Yellowhammers showed cereal fields as a minor but regular, especially in summer, feeding habitat. On average, winter cereal fields were selected to a lower portion as derived from the available portion in the birds' home ranges. Bird census data confirmed that Yellowhammers evidently prefer non-crop habitats over winter cereal fields. For risk assessment purposes a value for proportion of time spent foraging in winter cereal fields (PT) was 23.01% of their potential foraging time in cereal fields. The main food sources for Yellowhammers were arthropods (69 %), particularly Coleoptera, Diptera and Rhynchota. Cereal seeds amounted for 16 % of all food items detected.

Radio tracking of ten individual Quails showed that winter cereal fields provided the most important habitat for this species. The average Jacobs Index for Quails is positive, indicating a preference for winter cereals as a habitat (D = 0.39). Bird census data indicate that almost no other habitats than winter cereals and peas were used. For risk assessment purposes a value for proportion of time spent foraging in winter cereal fields (PT) was 64.27% of their potential foraging time in cereal fields. The Quail's diet was shown to consist mainly of arthropod material (mean 65.4 %), particularly Coleoptera and Dermaptera. Only 0.7 % of all detected food items were cereal seeds.

Radio tracking of three individual Tree Sparrows showed that the birds mainly fed in oilseed rape and winter cereal fields. The mean value of the Jacobs Index of D = -0.34 indicates that cereal fields were used less within the home range of the Tree Sparrows. Repeated bird census counts revealed that Tree Sparrows prefer the fruit stages of winter cereal growth (BBCH growth stages 7-9). For risk assessment purposes a value for proportion of time spent foraging in winter cereal fields (PT) was 21.71 % of their potential foraging time in cereal fields (data should be assessed with care due to the small sample size). The main food source for Tree Sparrows was plant material (mean 61.3 %) whereas arthropod material amounted for just 38.7 % on average. The biggest part of food items was made up by the category 'other seeds' (seeds, excluding cereal seeds) thus can be regarded as an important food source for Tree Sparrows. Cereal seeds accounted for 2.7 % of all detected food items.

Assessment and conclusion by applicant:

This ecological monitoring study was conducted to GLP but was not conducted according to a specific test guideline. However, this is typical of studies of this type therefore the study is still considered to be acceptable for regulatory purposes by supporting various aspects of the Bird & Mammal risk assessment.

The results of this study have been used as part of the refined risk assessment of the small omnivorous bird "lark" scenario, specifically to support the use of the skylark as a suitable focal species in cereal fields. Although the skylark was not the focus of the study, the results of the observations demonstrated that the skylark was the most abundant bird species found within cereal fields, thereby clearly demonstrating its suitability as a focal species for the refined risk assessment.

Data Point:	KCP 10.1.1.2/05
Report Author:	[redacted]
Report Year:	2008
Report Title:	Exposure of birds in cereals in Germany in spring - attractiveness of cereal fields, portion of time and diet composition
Report No:	RA06-006
Document No:	M-297061-01-1
Guideline(s) followed in study:	For the study there is no official test guideline available.
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and accepted RAR (2010)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

In this generic study a radio-tracking program was carried out in a typical cereal cultivating region in Germany in spring to obtain revised input data (PT and food choice) for refined exposure assessment. The skylark (*Alauda arvensis*) was chosen as the focal species because it is ground-foraging and focal to cereal fields. Radio tracking and analyses of faeces and stomach contents were used to quantify the importance of cereal fields as foraging habitats and to determine the diet composition of this species, respectively.

Additional information on the prevalence and abundance of other bird species in cereal fields and surrounding areas was gained by transect counts and scan sampling.

Eleven individually radio tracked skylarks showed no particular preference to, or avoidance of, cereal fields compared to other crops but spent a significant amount of their 'potential foraging time' in winter

cereal (BBCH 21 - 32) and in freshly sown spring cereal fields (BBCH ≤ 10). According to volume proportion analysis their diet mainly consisted of grass material and cereals, before spring cereals were drilled. Thereafter other plant material *i.e.* grass or cereal seeds and leaf remains made up the majority of the diet. Consumption of animal matter increased slightly during spring. For risk assessment purposes a value for portion of time (PT) spent potentially foraging in cereal fields for skylarks was calculated. For winter cereal fields in spring the PT value was 0.449 (44.9%), for freshly sown spring cereal fields 0.174 (17.4%).

Study area

The study was conducted in a typical agrarian landscape in the [redacted]. This region is a typical agrarian landscape, characterised by crop fields, meadows and surrounding woodland. Cultivation of winter and spring cereals is dominant and the skylark is prevalent. At the time the study was conducted, the majority of the winter cereal fields was in filling stages up to early stem elongation (BBCH 21-32) whilst the spring cereals were freshly sown (BBCH 00-10).

I. Methods

The study was carried out between March and June 2006.

Radio-tagging

The birds were captured on or in the direct vicinity of a cereal field. Trapping lots comprised seven winter cereal fields, two interspersed grasslands and one spring cereal field directly next to winter cereal fields (10-30 m distance).

Bird census transects

For the assessment of the bird community, frequency of occurrence (FO_{field}), dominance and abundance within winter and spring cereal fields compared to surrounding other crops and non-crop habitats, weekly transect counts of all birds present were conducted along three defined line transects including a range of habitats present in the study area. The cumulated length of all transects was 8,650 m with a total area of 82.3 ha.

Dominance denotes the percentage of individuals of a certain species compared to the total number of individuals of all species observed. To calculate the dominance parameter, the number of all individuals recorded for one species during all transect counts was divided by the sum of all individual numbers from all species.

To estimate abundance, the cumulated bird numbers counted during all transect counts were divided by the cumulated area of each habitat type during all transect counts, Thus, the data can be read as individuals per transect count per ha, or individuals per ha (as the numbers are standardised for one transect).

For calculation of the frequency of occurrence of a species the habitat of individual fields along the transect lines were summarised into three habitat categories (winter cereals, spring cereal and others). The frequency of occurrence was determined separately for each habitat category. FO_{field} is calculated as a percentage of the number of observations of a species for each survey across all three transect lines. Frequency of occurrence based on number of fields per habitat category was calculated for each survey and afterwards the mean across all surveys was determined (n=7 for both winter cereals and others; n=2 for spring cereals).

Scan sampling plots

Scan sampling was conducted on ten winter and ten spring cereal fields for one daylight period each. The total area across all scan sampling plots was 28.9 ha (14.6 ha for spring cereal fields and 14.3 ha for winter cereal fields).

Analysis of the scan sampling data includes calculation of dominance, abundance and frequency of occurrence (FO) for each recorded species with particular emphasis on species' behaviour.

Dominance denotes the percentage of individuals of a defined species compared to the total number of individuals of all species recorded during each session. Afterwards the mean abundance for each species was calculated over all scans performed during one session. Finally, the mean across all study plots was calculated for each species. Abundance data can be read as individuals per ha.

Frequency of occurrence denotes the percentage of scans with positive records of a defined species in relation to the total number of scans irrespective of the number of individuals recorded. FO was determined for each species and each session separately. For each species, the mean across all ten sessions was calculated for winter cereal and spring cereal fields separately.

As individual hazard would be overestimated if only abundance is taken into account, an additional analysis makes it possible to calculate the relative risk index (RR) (0 = no risk; 1 = high risk). To state the worst case scenario, the maximum relative risk index (RR_{max}) of all scan sampling sessions is reported.

Radio tracking of skylarks

Birds were trapped using a whoosh net (10 m × 4 m ×, 18 mm mesh) and tagged with radio transmitters. Individuals were attracted to the net using tape luring and a stuffed decoy. Within the study area a total of 13 birds were equipped with radio transmitters in and around the cereal fields (two skylarks disappeared after tagging and could not be radio tracked). Only tags of less than 5% of the bird's body weight were used to avoid adverse effects caused by tag-load. The tag used had a maximum weight of 1.2 g. The transmitters fell off after a few weeks.

All captured birds were marked with an aluminium ring with an engraved number to identify each bird. The birds were also marked with colour rings in order to enable recognition of individuals during subsequent visual contacts.

Individual birds were tracked continuously over an entire activity period (from dawn till dusk). Every change in behaviour and change of habitat or position within the same habitat was recorded. Special emphasis was placed on monitoring of habitat selection and feeding activities of skylarks, with a particular focus on the portion of time the birds spent foraging in cereal fields (PT). The following behaviour categories were used: 'foraging', 'reproductive behaviour' (*e.g.* singing, fighting, chasing conspecifics, incubating); 'active, possibly foraging' (*e.g.* moving signal, bird in locomotion); 'active, excluding foraging' (known details *e.g.* long flight, no foraging behaviour); 'inactive' (*e.g.* resting, sleeping, preening, with subcategories 'day' and 'night') and 'unknown' (no classification possible). The categories 'foraging', 'active, possibly foraging' and 'unknown' were summarised into one category called 'potentially foraging'.

The proportion of time foraging in cereal fields (compared with the total potential foraging time) was estimated from the data obtained by radiotracking and visual observation. These values are regarded as equivalent to the proportion of diet obtained from the treated area (PT).

Individual PT was calculated as:

Time potentially foraging in cereals
Time potentially foraging in all known habitats

To help interpret the PT values, the PT of each bird within cereal fields was compared with the total potential foraging time over all habitats for each radio tracking session. This comparison (calculated as the Jacobs' index [D]) illustrates the preference or avoidance of the individual bird for cereal fields as a feeding habitat during each tracking session.

Diet composition of skylarks

To gain information about the diet composition of the skylarks, sampling of faeces and/or stomach flushings were carried out.

To estimate the proportion of different food types in the diet (PD), faeces and/or stomach contents were sampled and analysed. The analytical results for the composition of faeces and stomach contents of each individual skylark were treated as independent events for calculating PD values for the skylark. For data analysis the volume proportion of each food item was calculated.

Additional observations

The whole study area was mapped for habitat types and crops.

The daily average temperature and daily precipitation data were obtained from the meteorological station of the "Deutscher Wetterdienst" (DWD) in Simmern-Wahlbach (non-GLP).

Information on the agriculture practices in the study fields were obtained from the respective farmers.

II. Results and Discussion

The average monthly temperatures for March (3.1°C) and April (8.2°C) did not deviate from the long-term average. The cumulated precipitation amounted to 87.5 mm collected in 22 days. Twenty-four days had no precipitation. Total precipitation in March (51.2 mm) and April (51.9 mm) was similar to the long-time average.

Tracking data

Radio tracking data revealed that the dominant behaviour recorded for individually tracked skylarks was 'potentially foraging' (47.9% of the time). Followed by 'inactive (night)' (41.8%) and 'active, excluding foraging' (7.1%). Birds 'inactive' during the day were only rarely recorded (3.1% of the time).

Home range and habitat selection

Radio tracked skylarks were often found in winter cereals (mean 51.1% of the time all tracking sessions included; mean 54.2% of the time without spring cereals available and 47.2% of the time when winter and spring cereals were available) but differences were obvious between individuals. The category 'other crops' was visited with a similar frequency (mean 48.9% of the time all tracking sessions included; 45.8% of the time without spring cereals available and 43.2% of the time when winter and spring cereals were available). Where available, spring cereals were visited less frequently (mean 9.6%). The standard deviations for each habitat category illustrate that the habitat choice differed considerably. Home range areas (minimum convex polygon) of individually tracked skylarks ranged from 3.8 – 36.4 ha (mean 10.7 ha). The average portion of winter cereals in these home range areas was 4.3 ha (40.0%).

Following the sowing of spring cereals, this crop was available in eight tracking sessions (mean 1.2 ha (10.7 %)). On average more than half of the area of recorded home ranges consisted of other crops and habitats (mean 5.5 ha for only winter cereals available and 6.4 ha for winter and spring cereals available, range 0.6 – 14.7 ha and 1.4 – 24.3 ha, median 3.1 ha and 3.2 ha, 90th percentile 12.6 ha and 14.0 ha, n = 10 and 8, respectively).

The results of the Jacobs Index show neither preference nor avoidance of cereals by the investigated skylarks. For winter cereal fields values ranged from complete avoidance (D = -1.0) to complete preference (D = 1.0, mean D = 0.0 ± 0.57, n = 18) and there were slightly more negative values than positive values. The situation was similar for spring cereals, where values ranged from D = -1.0 to D = 0.61 (mean D = -0.22 ± 0.72 n = 8). The mean Jacobs Index for cereals in general is D = 0.08.

Potential foraging time (PT values) in cereals

Eleven out of 13 radio tagged skylarks were tracked individually for one (n=4) and two (n=7) daylight periods. Two skylarks disappeared after tagging and could be radio tracked. Eighteen successful radio tracking sessions conducted were included in the analysis. Mean potential foraging times (PT) of skylarks are presented in the table below.

Table CP 10.1.1.2/05-1 Overview of PT values in cereal fields

Overview of the PT			
Proportion of time radio tracked skylarks spent potentially foraging (PT) in cereal fields in early spring of the total 'potential foraging' time			
Crop	Mean (%)	90% tile (%)	Tracking sessions (individuals)
Winter cereals (BBCH 21-32)	44.9	88.2	18 (11)
Spring cereals (BBCH 00-10, freshly sown)	17.4	41.8	8 (5)

Only one tracked skylark was not recorded 'potentially foraging' in any cereal field, despite three winter cereal fields in its home range. Seven skylarks were 'potentially foraging' in winter cereals only and five skylarks visited both winter and spring cereals.

Transect counts

Within the combined transect area of 823 ha a total of 1,210 individuals comprising of 32 species were recorded. Species composition, mean frequency of occurrence and abundance differed considerably between crops and adjacent non-crop habitats. Four species (skylark, carrion crow, yellowhammer and chaffinch) were recorded in all habitat categories. The recorded abundance of these species in winter and freshly sown spring cereals is presented in the table below.

Table CP 10.1.1.2/05-2 Bird abundance in cereal fields

Abundance of selected species after three transects counts[1] covering 576.0 ha				
Species	**Number of individuals in winter cereals**	**Abundance winter cereals (ind/count/ha)**	**Number of individuals in spring cereals**	**Abundance spring cereals (ind/count/ha)[1]**
Skylark	251	1.36	56	1.32
Yellowhammer	3	0.02	23	0.54
Chaffinch	58	0.32	13	0.31
Carrion crow	21	0.11	7	0.17
Grey partridge	12	0.07	-	0.00

[1]Based on cumulated bird numbers divided by the cumulated area of each habitat type

Of the 32 species, 14 were more or less frequently found in cereal fields with five species occurring in winter cereals only, one species in freshly sown spring cereals only and four species in both types (skylark, carrion crow, yellowhammer, chaffinch). The highest frequency of occurrence in winter and spring cereals was recorded for skylark (39.3% and 54.8%, respectively). Three species displayed dominance values in excess of 5.0% in winter and freshly sown spring cereals: skylark (70.9% and 52.3%, respectively), chaffinch (16.4% and 12.1%, respectively) and carrion crow (5.9% and 6.5%, respectively). The yellowhammer showed high dominance (21.5%) only in freshly sown spring cereals.

Table CP 10.1.1.2/05-3 Bird frequency of occurrence and dominance in cereal fields

Species	**Mean frequency of occurrence (%)**		**Dominance (%)**	
	Winter cereals	**Spring cereals**	**Winter cereals**	**Spring cereals**
Skylark	39.3	54.8	70.9	52.3
Carrion crow	1.3	6.2	5.9	6.5

Yellowhammer	0.3	17.1	0.9	21.5
Barn swallow	0.6	0	1.4	0
Grey partridge	0.6	0	3.4	0
Chaffinch	0.6	1.5	16.4	12.1

Frequency of occurrence from scan sampling

Generally, FO values for most species were higher in freshly sown spring cereals compared to winter cereals. Only song thrush and common buzzard showed higher mean FO in winter cereals. The highest mean FO per plot in freshly sown spring cereal fields was recorded for the skylark (43.6%), followed by the yellowhammer (42.5%), chaffinch (19.7%) and carrion crow (15.3%). In winter cereals the skylark also showed the highest mean FO (13.2%).

Table CP 10.1.1.2/05-4 Bird frequency of occurrence per scan in cereal fields

Species	FO winter cereals (%)	FO spring cereals (%)
Skylark	13.2	43.6
Yellowhammer	5.0	42.5
Chaffinch	2.7	19.7
Song thrush	1.3	0.6
Carrion crow	1.2	15.3
Common buzzard	0.6	0.1
Blackbird	0.5	2.2
Mistle thrush	0.4	0.9
Robin	0.5	0.2
Wood pigeon	0.3	6.3
Linnet	0.3	2.3
White wagtail	0.2	8.3

Dominance from scan scanning

The most dominant bird species in winter and spring cereal fields are presented in the table below. The four species dominant in winter cereals accounted for 85.2% of all sightings. The five dominant species in spring cereals accounted for 88.8% of all sightings.

Table CP 10.1.1.2/05-5 Bird dominance per scan in cereal fields

Species	Winter cereals (%)	Spring cereals (%)
Skylark	40.7	15.5
Yellowhammer	23.5	37.1
Chaffinch	13.7	17.7
Tree sparrow	-	11.1
Carrion crow	8.3	7.6

Calculation of the relative risk index RR (Fletcher & Greig-Smith 1988) showed none of the species at a particularly high risk. Again the highest values were found in freshly sown spring cereals, where the skylark (maximum RR = 0.389) showed the highest figure, followed by the corn bunting (RRmax =

0.321), yellowhammer (RRmax = 0.321), chaffinch (RRmax = 0.289), tree sparrow (RRmax = 0.231), white wagtail (RRmax = 0.178), carrion crow (RRmax = 0.133) and brambling (RRmax = 0.132). In winter cereals the highest value was recorded for the skylark (RRmax = 0.234) followed by the yellowhammer (RRmax = 0.156). All other species displayed relative risk indices below 0.1.

Diet composition (PD values)

Plant material dominated in the diet of sampled individuals, but there were clear differences between samples taken when only winter cereals were available and samples taken after drilling of spring cereals. In early spring skylarks feed mainly from true grass leaf remains, including cereals, and only less from seeds or plant material of other plants. Grass or cereal seeds did almost not appear in the birds' diet. After drilling of spring cereals skylarks seem to switch more to feeding from other plant material and true grass or cereal seeds. True grass/cereal leaf remains appeared in the birds' diet less frequently than before drilling time. Animal matter played a minor role in skylarks' diet in early spring but later on made up more than one-tenth of the samples volume. This may be explained by the increased arthropod population growth with rising temperature which allows the start of reproduction. The results of numerical proportion analysis show skylarks collecting a larger portion of arthropods in mid spring. The large portion of other plant seeds in skylarks' diet according to numerical proportion analysis in contrast to the results of the volume proportion calculation can be explained by the small size of these seeds.

Table CP 10.1.1.2/05-6 Diet composition values for skylarks in cereal fields

Overview of the PD		
Proportion of different food types in the diet (PD)		
Food items	Only winter cereals available – mean (%)	Winter and spring cereals available – mean (%)
Poaceae/cereal seeds	0.9	26.9
Poaceae/cereal leaf remains	69.7	31.3
Other seeds	12.7	5.6
Other plant material	1.5	23.5
Animal matter	3.1	12.8

[1]one transect count is defined as one transect, surveyed seven times

Conclusion

Ornithological observations (transect counts, scan sampling) confirmed that the skylark is the most abundant species on winter cereal fields in spring and also uses freshly drilled spring cereal fields as a foraging habitat. Eleven individually radio tracked skylarks showed no particular preference to, or avoidance of, cereal fields compared to other crops but spent a significant amount of their 'potential foraging time' in winter cereal (BBCH 21 - 32) and in freshly sown spring cereal fields (BBCH ≤ 10). According to volume proportion analysis their diet mainly consisted of grass material and cereals, before spring cereals were drilled. Thereafter other plant material *i.e.* grass or cereal seeds and leaf remains made up the majority of the diet. Consumption of animal matter increased slightly during spring. For risk assessment purposes a value for portion of time (PT) spent potentially foraging in cereal fields for skylarks was calculated. For winter cereal fields in spring the PT value was 0.449 (44.9%), for freshly sown spring cereal fields 0.174 (17.4%).

Assessment and conclusion by applicant:

This ecological monitoring study was conducted to GLP but was not conducted according to a specific test guideline. However, this is typical of studies of this type therefore the study is still considered to be acceptable for regulatory purposes by supporting various aspects of the Bird & Mammal risk assessment.

The results of this study have been used as part of the refined risk assessment of the small omnivorous bird "lark" scenario, specifically to support the use of the skylark as a suitable focal species in cereal fields.

The study demonstrated that the skylark was the most abundant bird species found in cereals fields. 90th percentile PT values of 0.882 and 0.418 were determined for the skylark in winter cereals at BBCH 21-32 and freshly sown spring cereals, respectively. These PT values have been used as part of an assessment from multiple studies to derive a refined PT value for risk assessment (refer to M-557330-01-1).

Data Point:	KCP 10.1.1.2/06
Report Author:	[redacted]
Report Year:	2006
Report Title:	Bird species in cereal fields in [redacted] field data for the determination of focal species
Report No:	RA05-224/2
Document No:	M-291779-01-1
Guideline(s) followed in study:	EU Council Directive 91/414/EEC amended by the Commission Directive 96/68/EC; SANCO 4145/2000
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and accepted RAR (2010)
GLP/Officially recognised testing facilities:	No, not conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The objectives of this generic study were to determine the qualitative and quantitative composition of the bird community employing the parameters, frequency of occurrence (FO_{field} and FO_{survey}) and dominance as overall or cereal growth stage specific descriptors, respectively. Another objective was to then allocate the selected species to defined foraging guilds, diet guilds and size classes.

The results of the study are summarised as a list of candidates for focal bird species.

I. Study Design

The objectives of this generic study were to determine the qualitative and quantitative composition of the bird community employing the parameters, frequency of occurrence (FO_{field} and FO_{survey}) and dominance as overall or cereal growth stage specific descriptors, respectively. Another objective was to then allocate the selected species to defined foraging guilds, diet guilds and size classes.

The test system consisted of 21 commercial cereal fields with standard husbandry according to good agricultural practise (GAP) in [redacted]

Cereal fields were visited between middle of March and the beginning of May 2006. Three surveys were conducted in every field within a two month period. The avifauna of the cereal fields was surveyed by the line transect method. To meet the specific methodological requirements, the line transect method described by Bibby *et al.* (1992) was adapted for this study.

All bird species were recorded in each cereal field by walking slowly along a defined longitudinal line transect, allowing a clear view between the rows of cereal plants. The length of the line transects was defined by the length of the field. Each of the individual birds visually or acoustically registered was assigned to one of two areas.

Only birds present (foraging, roosting, singing) in the 'in-crop transect band' of each cereal field were included for data analysis. Birds flying up to a height of 5 m above average crop height (*e.g.* actively hunting swallows, swifts or raptors) were also included in the analysis. Birds not directly associated with the cereal field, *e.g.* flying above 5 m over average crop height, were assigned to the 'outside transect band' and ignored for the purposes of this analysis.

Frequency of occurrence

The frequency of occurrence (FO) can be determined in two different ways, related to the number of fields and related to the total number of surveys.

FO_{field} denotes the number of fields in which a defined species was recorded, given as percentage of the total number of fields regardless of the number of individuals observed. This approach serves as a measure for the spatial frequency of occurrence.

FO_{survey} denotes the number of surveys in which a defined species was recorded, given as percentage of the total number of surveys. This approach gives an approximation for the temporal evenness of occurrence throughout the complete study period.

Dominance

The dominance denotes the relative occurrence of bird species within the bird community. It is reported as the percentage number of individuals of the respective species compared to the total number of individuals throughout all species (calculated as arithmetic means over all cereal fields). Number of individuals of a given bird species in all 21 cereal fields analysed. A species was termed 'dominant' when the dominance value of the species was greater than or equal to 5%.

Flocking behaviour

Dominance data may be biased by species with flocking behaviour. A method of aggregation was performed to separate between flocking and non-flocking species across and during different survey periods. To obtain this information, the number of individual bird contacts was plotted against the number of surveys, in which the respective species had been detected. To categorise flocking species and/or species with numerous occurrences a threshold value of on average more than five individual bird contacts per survey was chosen. For bird species occurring only in small groups values between on average 3 5 contacts per survey were chosen, whereas non-flocking species (rare or uniformly distributed species) were defined by values of on average less than three contacts per survey.

II. Results and Discussion

During the course of the study, a total of 317 individual bird contacts, comprising 21 different bird species, was recorded within the 'in-crop transect band' of the 21 cereal fields in [redacted].

The highest FO_{field} (85.7%) across cereal fields was recorded for the corn bunting, followed by the yellow wagtail (71.4%), calandra lark (52.4%), fan-tailed warbler (52.4%), meadow pipit (42.9%), crested lark (33.3%), quail (28.6%), red-legged partridge (28.6%) and barn swallow (23.8%).

The highest time-weighted occurrence throughout the study period, as indicated by the FO_{survey} was recorded for the corn bunting (63.5%), followed by the yellow wagtail (47.6%), calandra lark (28.6%), fan-tailed warbler (27.0%), crested lark (14.3%) and meadow pipit (14.3%).

The relative consistency of occurrence is noted for the corn bunting over all three growth stages, while occurrence is more variable for other species *e.g.* quail, barn swallow, marsh harrier, *etc*.

The highest dominance value was recorded for the yellow wagtail (27.4%), followed by the corn bunting (22.4%), calandra lark (14.2%), fan-tailed warbler (8.2%) and red-legged partridge (5.0%). These five species were responsible for 77.2% of all sightings.

Table CP 10.1.1.2/06-1 Frequency of occurrence and dominance of bird species in relation to the total number of study plots in cereal fields in Andalusia (southern Spain)

Species	FO_{field} [%] (n = 21)	FO_{survey} [%] (n = 63)	Dominance [%]
Corn bunting (*Miliaria calandra*)	85.7	63.5	22.4
Yellow wagtail (*Motacilla flava*)	71.4	47.6	27.4
Calandra lark (*Melanocorypha calandra*)	52.4	28.6	14.2
Fan-tailed warbler (*Cisticola juncidis*)	52.4	27.0	8.2
Meadow pipit (*Anthus pratensis*)	42.9	14.3	3.5
Crested lark (*Galerida cristata*)	33.3	14.3	4.1
Quail (*Coturnix coturnix*)	28.6	9.5	2.2
Red-legged partridge (*Alectoris rufa*)	28.6	9.5	3.0
Barn swallow (*Hirundo rustica*)	23.8	7.9	4.7
Montagu's harrier (*Circus pygargus*)	14.3	6.3	1.4
Skylark (*Alauda arvensis*)	14.3	4.8	0.9
Collared pratincole (*Glareola pratincola*)	9.5	3.2	0.6
European bee-eater (*Merops apiaster*)	9.5	3.2	1.3
Mallard (*Anas platyrhynchos*)	9.5	3.2	0.9
Marsh harrier (*Circus aeruginosus*)	9.5	3.2	0.6
Kestrel (*Falco tinnunculus*)	4.8	1.6	0.3
Lesser kestrel (*Falco naumanni*)	4.8	1.6	0.3
Little bustard (*Tetrax tetrax*)	4.8	1.6	0.3
Little owl (*Athene noctua*)	4.8	1.6	0.6
Short-toed lark (*Calandrella brachdactyla*)	4.8	1.6	0.3
Stone curlew (*Burhinus oedicnemus*)	4.8	1.6	0.6

List of candidates of focal bird species

Nine species were found to be characterised by an FO_{field} of at least 20%. The majority of these species were grouped into the small bird category (< 50 g), while only three medium-sized species (50 – 500 g) were recorded in this category (calandra lark, quail, redlegged partridge).

Table CP 10.1.1.2/06-2 List of focal species in cereal fields in Andalusia (southern Spain)

Species	FO_{field} [a) b)] [%]	FO_{survey} [a) c)] [%]	Dominance [a) d)] [%]	Body weight [e)] [g]	Stratum use [f)]	Diet guild [g)]
Corn bunting (*Miliaria calandra*)	85.7	63.5	22.4	46.0	Ground	Omnivorous
Yellow wagtail (*Motacilla flava*)	71.4	47.6	27.4	17.6	Ground	Insectivorous

Species	FO_{field} a) b) [%]	FO_{survey} a) c) [%]	Dominance a) d) [%]	Body weight e) [g]	Stratum use f)	Diet guild g)
Calandra lark (*Melanocorypha calandra*)	52.4	28.6	14.2	61.6	Ground	Omnivorous
Fan-tailed warbler (*Cisticola juncidis*)	52.4	27.0	8.2	6.6	Foliage	Insectivorous
Meadow pipit (*Anthus pratensis*)	42.9	14.3	3.5	18.4	Ground	Insectivorous
Crested lark (*Galerida cristata*)	33.3	14.3	4.2	39.0	Ground	Omnivorous
Red-legged partridge (*Alectoris rufa*)	28.6	9.5	5.0	391.0	Ground	Omnivorous
Quail (*Coturnix coturnix*)	28.6	9.5	2.2	90.0	Ground	Omnivorous
Barn swallow (*Hirundo rustica*)	23.8	7.9	4.7	15.8	Aerial	Insectivorous

a) Across the complete study period (3 survey periods)

b) Based on 21 study plots (cereal fields)

c) Based on 63 surveys

d) Based on the arithmetic mean of the number of individuals per study plot of one species in relation to the mean number of individuals per study plot of all species for a total number of 21 fields

e) According to Dunning (1993). In case sex-specific figures were provided, the lower number was chosen

f) Predominant foraging stratum during growing season according to Perrins (1998)

g) Predominant diet composition during growing season according to Perrins (1998)

III. Conclusion

In this generic study the frequency of occurrence was used to determine a list of candidates of focal bird species in cereal fields in [redacted] that could be used for assessing the risk of plant protection products to wild bird species in a refined assessment. The frequency of occurrence (FO_{field}; FO_{survey}) and dominance were considered to be the decisive parameters for the derivation of focal species at a given period of time. The derivation of these parameters was based on both visual and acoustic identification of bird species. Due to this form of appraisal, there is potential for recording bias towards conspicuous species (loud song, *e.g.* skylark) which might be recorded more frequently compared to more elusive species (inconspicuous song/calls, *e.g.* linnet), which might be underrepresented. Indeed, the detectability of a species decreases with increasing distance from the observer - particularly for inconspicuous species – and for this reason, the data analysis is restricted to birds recorded within the 100 m 'in-crop transect band' (50 m to either side of the observer) where this bias is negligible (Bibby *et al.* 1992).

As noted earlier, the cut-off criterion for FO_{field} (≥ 20%) was defined arbitrarily. Despite this, the list of candidates of focal bird species derived by using these cut-off criteria generally contain the most abundant bird species.

The major criteria for selection of candidate focal species was the FO_{field} value. The ranking of species based on FO_{survey} showed no differences compared to FO_{field} values. In general, the ranking of species was similar for all parameters calculated in this study (FO_{field}, FO_{survey}, dominance).

The FO_{field} value describes the likelihood of a defined species to occur on any particular field, *i.e.* species with high FO_{field} values are more likely to be exposed to crop protection products used in cereal fields at certain times and are therefore relevant for risk assessments.

The FO_{survey} is more indicative of time-weighted occurrence (opposed to spatial as described by FO_{field}). This value is usually also high for species with high FO_{field} values, but differences, caused by seasonal aspects are not detectable here. For example, a low FO_{survey} may be based on the occurrence of a species restricted to one or two crop stages only but over a large number of fields (high FO_{field}), as in the case of migratory species, *e.g.* barn swallow. On the other hand a species may occur throughout the season but only in a limited number of fields, resulting in a low FO_{survey} and a low FO_{field}.

The dominance value requires careful interpretation since it may be biased by the flocking behaviour of some species. Species that exhibit only temporary flocking behaviour achieve high dominance values while the FO is rather low. To shed some light on this aspect the number of surveys in which a species was recorded is plotted against the number of recorded individuals of the same species for each survey period. Three categories were distinguished: 1) species occurring with on average less than three individuals per survey (scarce species), 2) species occurring with on average 3 – 5 individuals per survey (small groups, family party) or 3) species occurring with on average more than five individuals per survey (flocking species). Particularly these latter species are higher ranked according to dominance values than with respect to frequency of occurrence (FO_{field}, FO_{survey}).

FO_{field} values were given more significance than FO_{survey} or dominance values. However, FO_{survey} values can indicate the importance of the crop for a particular species across several growth stages (time-weighted frequency of occurrence) and the dominance value will help to identify flocking species compared to rare species. Therefore, the selection of focal species as presented in this report is justified and provides a good overview of the bird species occurring regularly in cereal fields in Andalusia (southern Spain).

Assessment and conclusion by applicant:

This ecological monitoring study was not conducted to GLP nor was it conducted according to a specific test guideline. However, this is typical of studies of this type therefore the study is still considered to be acceptable for regulatory purposes by supporting various aspects of the Bird & Mammal risk assessment.

The results of this study have been used as part of the refined risk assessment of the small omnivorous bird "lark" scenario, specifically to support the use of the skylark as a suitable focal species in cereal fields. In this study the skylark was found to be present in the monitored cereals field and, although not the most abundant species, the study confirms that skylarks do visit cereal fields in Spain.

Data Point:	KCP 10.1.1.2/35
Report Author:	[redacted]
Report Year:	2016
Report Title:	Calculation of 21 day PT values for skylarks in winter cereals in spring
Report No:	M-557330-01-1
Document No:	M-557330-01-1
Guideline(s) followed in study:	None
Deviations from current test guideline:	None
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	not applicable
Acceptability/Reliability:	Yes

Executive Summary

Empirical PT data are available for individual skylarks from several radio-tracking studies with similar statistics including cereal fields in the UK, Germany and Austria (Wolf 2005, Moosmayer 2008, Prosser 2010).

The radio-tracking data of 52 tracking sessions from 33 individual skylarks from three studies were pooled to estimate 21 day-PT values via Monte Carlo simulations.

In summary, Monte Carlo simulations offer a suitable tool to derive more realistic PT values from single radio-tracking data in consideration of intra-individual variation of daily PT. Simulations of 1000 MC individuals with 17 days each seem to provide stable estimates of PT. Therefore, the estimated 90th percentile PT of 0.487 from 10000 MC individuals with 21 days based on 52 empirical PT data from 33 individual skylarks has to be considered as still conservative but more realistic compared to the daily PT of 0.934 calculated directly from the empirical data.

The modelled 90th percentile 21-d PT is much closer to the mean of the daily PT (*i.e.* 0.317) than to the 90*th* percentile of the daily PT data (i.e. 0.934).

I. Materials and Methods

Empirical PT data are available for individual skylarks from several radio-tracking studies with similar statistics including cereal fields in the UK, Germany and Austria (Wolf 2005, Moosmayer 2008, Prosser 2010). Variances of the three data sets 0.103 (GER), 0.092 (UK) and 0.123 (AU) showed no significant differences as indicated by t-test characteristics (one-sided, similar variance, $t = 0.097–1.284$, $p > 0.05$). For each individual ($n = 33$) 1 -4 measured PT values are available. The measurements represent the portion of time when tracked birds were active (*i.e.* potentially foraging) on cereal fields.

Table CP 10.1.1.2/35-1 Measured empirical PT data for skylarks potentially foraging in post-emergence cereal fields from radio-tracking studies in Austria, Germany and the UK in the spring

Country	Individual No.	Measured PT (one day)	Source	Country	Individual No.	Measured PT (one day)	Source
Germany	4	0.760	Moosmayer 2008	Austria	1a	0.010	Wolf 2005
Germany	6	0.534		Austria	1b	0.014	
Germany	12	0.402		Austria	10a	1.000	
Germany	8a	0.325		Austria	10b	0.989	
Germany	10a	0.317		Austria	11a	0.080	

Country	Individual No.	Measured PT (one day)	Source	Country	Individual No.	Measured PT (one day)	Source
Germany	10b	0.567		Austria	13a	0.103	
Germany	11a	0.863		Austria	13b	0.100	
Germany	11b	0.383		Austria	13c	0.023	
Germany	13a	0.034		Austria	14a	0.008	
Germany	13b	0.039		Austria	14b	0.000	
Germany	1a	0.032		Austria	15a	0.964	
Germany	1b	0.149		Austria	15b	0.653	
Germany	2a	1.00		Austria	18a	0.051	
Germany	2b	0.927		Austria	2a	0.223	
Germany	8b	0.583		Austria	2b	0.649	
Germany	9a	0.844		Austria	4a	0.103	
Germany	9b	0.317		Austria	4b	0.234	
UK	NJ46289	0.458		Austria	7a	0.935	
UK	NJ46375	1.000		Austria	8a	0.345	
UK	NN69262	0.673		Austria	8b	0.638	
UK	NN69263	0.242		Austria	8c	0.242	
UK	NN69264	0.296	Prosser 2010	Austria	8d	0.079	
UK	NN69268	0.070		Austria	9a	0.324	
UK	NN69269	0.124		Austria	9b	0.123	
UK	NN69270	0.444		Austria	9c	0.567	
UK	VA64816	0.025					
UK	VV01912	0.173					

The comparability of intra- and inter-individual variability is based on visual evaluation of the empirical data distribution as well as ecological and biological knowledge for skylarks according to Ludwigs *et al*. (2015). Finally, 52 empirically measured PT values of 33 individual skylarks were pooled for the Monte Carlo simulation.

The default period for assessing the long-term exposure of individuals potentially at risk from pesticide applications is 21 days (EFSA 2009). Multi-day empirical PT values of single individuals show empirical differences between different days of tracking. The average of such empirical daily PT values over time represents a realistic long-term value reflecting the average behaviour of this individual.

Since the protection goal for assessing risks *via* pesticide use for birds and mammals is the population rather than the individual level (EFSA 2009, 2010), the assessment of the PT should include several individuals. An appropriate methodology to estimate such a PT value is the use of Monte Carlo (MC) simulations (Manly 2007; cf. also Wang 2014). To this aim, repeated calculations were performed *via* a computing approach randomly combining the empirical data to generate 21-day radio tracking datasets for a defined number of virtual individuals. The resulting output values for each calculation reflect the variability of all input values. Regarding PT determination, this means that a large number of individuals (n = 10000) is simulated, each represented by random combination of empirical PT values *via* bootstrapping describing the average behaviour. These model individuals represent the population, *i.e.*

the protection goal. The selection of measured values for each simulated Monte Carlo individual is critical for a realistic approximation of a long-term PT. The Monte Carlo simulations were based on 10000 Monte Carlo individuals, *i.e.* 10000 rows, each comprising 21 single PT values, each row representing a long-term 21-day PT of one single Monte Carlo individual.

Since a period of 21 days is considered an appropriate standard default period for long-term risk assessments according to EFSA (2009) that covers the average long-term behaviour of an individual, the arithmetic mean PT over the 21 day period is calculated for each individual. Finally, to obtain a realistic worst-case PT value that protects 90 percent of the population, the 90th percentile is calculated from the derived mean values per individual (Ludwigs *et al.* 2015). For comparison the 90th percentile and mean of the empirical daily PT values are presented. Monte Carlo simulations on the same data set of 52 empirical data were conducted with 50, 100, 1000 and 10000 MC individuals, respectively. As can be seen from the comparison of these data sets, results are very similar and particularly stable from 1000 individuals onwards. It is therefore considered, that the results based on 10000 MC individuals represent a robust estimate of the expected 21-day PT distribution for skylarks in winter cereals during spring.

II. Results and Discussion

The radio-tracking data of 52 tracking sessions from 33 individual skylarks from three studies were pooled to estimate 21 day-PT values via Monte Carlo simulations. As a result of the simulations, a long-term 21-day 90th percentile PT of 0.487 (mean 0.392) was derived. In contrast, the 90th percentile daily PT value is 0.934 (mean 0.317).

Table CP 10.1.1.2/35-2 Measured daily PT and 21-d PT from Monte Carlo (MC) simulations for skylarks in cereal fields

Parameter	21-d PT (MC simulations)				Daily PT (empirical data)
	50 ind.	100 ind.	1000 ind.	10000 ind.	(n = 52)
90th percentile	0.500	0.477	0.488	0.487	0.934
Arithmetic mean	0.404	0.392	0.391	0.392	0.317

In order to determine real 21-day PT values as recommended by EFSA (2009), a large number of individual birds (20 or more) would have to be radio-tracked for 21 full daylight periods each. Such studies are currently not available for the potential focal species as recommended by EFSA (2009) or proposed by Dietzen *et al.* (2014). An alternative option to determine appropriate 21-day long-term PT values is to model PT values of a population based on empirically recorded full day PT data *via* Monte Carlo simulations (Wang 2014, Ludwigs *et al.* 2015). This approach was used to derive a long-term 21-day PT value for skylarks based on radio-tracking data of 33 individual skylarks tracked over 52 sessions in cereal fields. Monte Carlo simulations of 52 empirical PT data (10000 MC individuals) yielded a 90th percentile 21 day PT of 0.487, which is clearly lower than the 90th percentile PT of 0.934 of the daily PT values. The main reason for the discrepancy between the results from different approaches is the lack of consideration of intra-individual variation of PT values over time for individuals (cf. Wang 2014, Ludwigs *et al.* 2015). This means, the variations of PT from one day to another for individual birds is not appropriately reflected by the empirical PT values for one individual on just one day. For example, a skylark radio-tracked on four different days showed daily PT values between 0.079 and 0.638 and similar intra-individual variation has to be expected for the whole population. This is supported by data from other species (Finch *et al.* 2006, Barfknecht *et al.* 2007, Ludwigs 2009, Ludwigs *et al.* 2015). Monte Carlo simulations take into account these intra-individual day by day variations and provide a more realistic estimate of PT.

Single measurements of daily PT values clearly overestimate the portion of time individual birds on average spend potentially foraging in cereal fields due to the strong proportional influence of extreme values. This influence decreases with increasing number of repeated radio-tracking (multiday tracking)

of individuals due to inclusion of intra-individual variation from day to day. With at least 17 days of averaging time, mean PT values for the MC individuals become fairly stable with only minor fluctuations.

III. Conclusion

In summary, Monte Carlo simulations offer a suitable tool to derive more realistic PT values from single radio-tracking data in consideration of intra-individual variation of daily PT. Simulations of 1000 MC individuals with 17 days each seem to provide stable estimates of PT. Therefore, the estimated 90th percentile PT of 0.487 from 10000 MC individuals with 21 days based on 52 empirical PT data from 33 individual skylarks has to be considered as still conservative but more realistic compared to the daily PT of 0.934 calculated directly from the empirical data.

The modelled 90th percentile 21-day PT is much closer to the mean of the daily PT (*i.e.* 0.317) than to the 90th percentile of the daily PT data (*i.e.* 0.934).

Assessment and conclusion by applicant:

This report presents the results of an exercise conducted in order to derive PT values for skylarks in cereal fields for use in a refined risk assessment. The radio-tracking data of 52 tracking sessions from 33 individual skylarks from three studies, including M-297061-01-1 discussed above, were pooled to estimate 21 day-PT values for the skylark in winter cereals in Spring. A 90th percentile PT value of 0.487 was determined and this value has been applied to the refined risk assessment.

The results are considered to be valid and acceptable for use in the risk assessment.

For procedural reasons studies listed in the Table CP 10.1.1.2-1 below are included in the current dossier as available data or information previously submitted but not necessarily evaluated. However, these reports have been fully superseded by newer studies. Consequently, no summaries of the reports have been included in the dossier.

Table CP 10.1.1.2-1: Studies previously submitted and not relied upon for the risk assessment

Data Point	Document No.	Date	Title
KCP 10.1.1.2/07	M-103906-01-1	2002	AGROBIRD - Database 2002 - Motacilla flava (Linnaeus, 1758)
KCP 10.1.1.2/08	M-091051-01-1	1997	Habitatwahl, Habitatnutzung und Bruterfolg der Schafstelze Motacilla flava in einer Agrarlandschaft
KCP 10.1.1.2/09	M-091022-01-1.	1993	Die Brutpopulation der Schafstelze Motacilla flava im unteren Thurgau und im angrenzenden Zuercher Weinland
KCP 10.1.1.2/10	M-090336-02-1	2005	Generic field monitoring of birds in potato cultivation in northern Germany
KCP 10.1.1.2/11	M-048098-02-1	2002	Methods for estimating daily food intake of wild birds and mammals
KCP 10.1.1.2/12	M-263242-01-1	2005	Review on initial residue levels of pesticides in arthropods sampled in field studies
KCP 10.1.1.2/13	M-281566-01-1	2006	Determination of Spiroxamine residues in the carabid beetle Poecilus cupreus L. - extended laboratory study -
KCP 10.1.1.2/14	M-288174-01-2	2007	Determination of the residues of spiroxamine (KWG 4168) in/on insects after application with spiroxamine 750 g a.s./ha
KCP 10.1.1.2/15	M-042192-01-1	2002	JAU 6476 480 SC - Magnitude of residue in/on wheat forage, a potential wildlife feed item

KCP 10.1.1.2/16	M-290596-01-1	2007	Metabolite JAU 6476-desthio : Determination of effects on carbon transformation in soil
KCP 10.1.1.2/17	M-108441-01-1	2002	AGROBIRD - Database 2002 - Turdus merula
KCP 10.1.1.2/18	M-111045-01-1	1978	Factors affecting the diet of farmland skylarks, Alauda arvensis
KCP 10.1.1.2/19	M-115925-01-1	1998	The complete birds of the western palearctic - selected chapters about the following species: Wren - Troglodytes troglodytes
KCP 10.1.1.2/20	M-304972-01-1	1993	CRC Handbook of Avian Body Masses
KCP 10.1.1.2/21	M-256356-01-1	2005	The ecological relevance of common voles (Microtus arvalis) in cereal field as indicator species for the exposure of plant protection products
KCP 10.1.1.2/22	M-078001-01-1	2002	AGROBIRD - Database 2002 - Coturnix coturnix
KCP 10.1.1.2/23	M-087189-01-1	1990	Handbuch der Voegel Mitteleuropas - Saatgans
KCP 10.1.1.2/24	M-279533-01-1	1989	Beziehungen zwischen Vogelwelt und Vegetation im Kulturland, Untersuchungen im suedwestdeutschen Huegelland - Beihefte zu den Veroeffentlichungen fuer Naturschutz und Landschaftspflege in Baden-Wuerttemberg Auszug: Feldlerche und Zaunkoenig
KCP 10.1.1.2/25	M-285584-01-1	2006	Bekanntmachung Nr. 06/02/26 ueber die Umsetzung des EU-Guidance Document fuer Voegel und Saeuger Location: Germany
KCP 10.1.1.2/26	M-001046-01-1	1977	Prey selection and social behaviour in wagtails (Aves: Motacillidae)
KCP 10.1.1.2/27	M-001033-01-2	1984	Die Schafstelze - Motacilla flava
KCP 10.1.1.2/28	M-087193-01-1	1998	Goose damage to grassland and winter cereals by white-fronted and bean geese (Anser albifrons and A. fabalis) in the lower rhine area, Germany

CP 10.1.2 Effects on terrestrial vertebrates other than birds

The available mammalian toxicity data for spiroxamine and Spiroxamine EC 500 are summarised in the table below.

Table CP 10.1.2-1 Summary of mammalian toxicity studies with spiroxamine

Organism	Test item	Test type	Endpoints		Reference
Rat	Spiroxamine	Acute oral toxicity	LD_{50} 595 mg a.s./kg bw (male) LD_{50} >500<560 mg a.s./kg bw (female)	EU	M-007791-01-1
Mouse	Spiroxamine	Acute oral toxicity	LD_{50} **460 mg a.s./kg bw** (male) LD_{50} 561 mg a.s./kg bw (female)	EU	M-007804-01-1

Organism	Test item	Test type	Endpoints		Reference
Rat	Spiroxamine	Chronic, 2-generation	NOAEL (parental) ♂/♀ 5.5 / 6.7 mg a.s./kg bw/day NOAEL (reproductive) ♂/♀ **21.0** / 21.2 mg a.s./kg bw/day NOAEL (offspring) ♂/♀ 6.5 / 6.7 mg a.s./kg bw/day	EU	M-304231-01-1

EU: previously evaluated as part of the original EU review and listed in EFSA conclusion and DAR
Values in **bold** have been used in the risk assessment

The available mammalian toxicity data for prothioconazole and prothioconazole-desthio are summarised in the table below.

Table CP 10.1.2-2 Summary of mammalian toxicity studies with prothioconazole and prothioconazole-desthio

Organism	Test item	Test type	Endpoints		Reference
Rat	Prothioconazole	Acute oral toxicity	**LD_{50} > 6200 mg a.s./kg bw**	EU	EFSA Conclusion[1]
Rat	Prothioconazole	Chronic, 2-generation	$NOEL_{parental}$ 9.7 mg a.s./kg bw/day **$NOEL_{repro}$ 95.6 mg a.s./kg bw/day**	EU	
Mouse	Prothioconazole-desthio	Acute oral toxicity	**LD_{50} 2235 mg/kg bw (male)** LD_{50} 3459 mg/kg bw (female)	EU	
Rat	Prothioconazole-desthio	Acute oral toxicity	LD_{50} 2806 mg/kg bw (male) LD_{50} 2506 mg/kg bw (female)	EU	
Rat	Prothioconazole-desthio	Chronic, 2-generation	$NOEL_{parental}$ 2.5 mg/kg bw/day **$NOEL_{repro}$ 10 mg/kg bw/day**	EU	

EU: previously evaluated as part of the original EU review and listed in EFSA conclusion and DAR
[1] EFSA Scientific Report (2007) 106, 1-98. Conclusion on the peer review of prothioconazole
Values in **bold** have been used in the risk assessment

The available mammalian toxicity data for Prothioconazole + Spiroxamine EC 460 are summarised in the table below.

Table CP 10.1.2-3 Summary of mammalian toxicity studies with Prothioconazole + Spiroxamine EC 460

Organism	Test item	Test type	Endpoints		Reference
Rat	Prothioconazole & Spiroxamine EC 460	Acute oral toxicity	**LD_{50} 750 mg product/kg bw (mean)**	EU	M-087810-02-1

EU: previously evaluated as part of the original EU review and listed in EFSA conclusion and DAR
Values in **bold** have been used in the risk assessment

Toxicity endpoints for risk assessment

The acute risk assessment for spiroxamine has used the lowest available LD_{50} value which is 460 mg a.s./kg bw which was determined in male mice.

For the reproductive risk assessment of spiroxamine an ecotoxicologically relevant endpoint has been determined. According to the outcome of the pesticides peer review meeting on recurring issues in ecotoxicology (EFSA PPR meeting 133, September 2015), an ecotoxicologically relevant endpoint should be set in collaboration with mammalian toxicologists and should be used in all the steps of the risk assessment.

Report M-762441-01-1 presents an assessment of the available mammalian toxicology data with spiroxamine along with a summary of the specific effects seen for various parameters for each study. A NOAEL of 21.0 mg a.s./kg bw/day has been determined from the rat two generation study and considered suitable for ecotoxicological risk assessment of wild mammals. The refinement is based on the assumption that the effects reported at the 300 ppm top dose level do not influence the population success and the total reproductive outcome of mammals in treated areas. At this dose parental animals showed slight decreases of body weight (up to 8.3 %) or body weight gain (up to 14.2 %) as well as irritation induced hyperkeratosis of the oesophagus epithelium. There were delays to developmental milestones of reaching puberty, i.e. preputial separation (PPS) in males and vaginal opening (VO) in females, in the F_1 offspring only which were apparently treatment related, but these were relatively small and are not considered to have an adverse effect at the population level. It is noted that in the same study the reproductive parameters (mating, fertility, oestrous cycling, sperm motility, sperm count, sperm morphology, pregnancy, natural delivery, litter observations, mean ovarian follicles, *corpora lutea*) were unaffected at the highest dose therefore it has been demonstrated that these small delays in PPS and VO do not have an adverse effect on the parameters that are considered to be relevant at the population level.

Thus, the lowest ecotoxicologically relevant NOAEL, suitable for use in the mammalian reproductive risk assessment, was considered to be 21.0 mg a.s./kg bw/day. Details of the assessment can be found in report M-762441-01-1 which has been summarized at the end of this section.

Literature paper M-662216-01-1 presents the results of population modelling conducted in order to assess the impact of body weight effects on the population development of the common vole. The study revealed that there was no detectable influence of common vole body weight on the reproductive success and survival during most times of the year and that reproductive success was mainly influenced by the date of birth. This study supports the position that the relatively small reductions in body weight recorded in the rat two-generation study are unlikely to have an adverse effect at the population level and are therefore not ecotoxicologically relevant.

The NOAEL of 21.0 mg a.s./kg bw/day has been used in all tiers of the reproductive risk assessment for spiroxamine.

For prothioconazole the endpoints that have been presented in the 2007 EFSA Conclusion have been used without further consideration. Discussion over the endpoints for prothioconazole is not considered to be part of the Renewal of Approval for spiroxamine.

Metabolites

Numerous metabolites of spiroxamine are formed in plants following application to crops. In the Toxicology and Residues sections of the dossier the spiroxamine metabolites have been categorised into three distinct groups (Group A, B and C, respectively). Toxicology dara are available for several of these metabolites. Metabolite M13 has been used to represent all Group B metabolites therefore the toxicology data generated using this metabolite is also considered to cover the plant metabolites M35 and M36. Likewise, M28 has been used to represent all Group C metabolites therefore the toxicology data generated using this metabolite is also considered to cover the plant metabolites M29, M30 and M31. It is also noted that Group A metabolites are considered to be covered by parent spiroxamine.

The table below presents each plant metabolite along with the percentage TRR and actual residue value from the crop metabolism studies. Available toxicology data have also been presented as well as an indication of whether or not each plant metabolite was also found in the animal metabolism studies on laying hen, rat and goat. Finally, an assessment is made regarding the relevance of each plant metabolite to the risk assessment. Only metabolites which were formed in plants at ≥10% TRR are considered to be potentially relevant to the bird and mammal risk assessment.

Note that only metabolites which were found in the crop metabolism studies have been presented below, however M13 and M13-acetate have been included in the table below because there are toxicology data which are relevant to other Group B plant metabolites.

Table CP 10.1.2-4 Assessment of potential exposure of mammals to metabolites of spiroxamine formed in plants

Plant Metabolite	Maximum levels of residue in plants	Metabolite found in animal studies?	Mammalian toxicity data available?	Conclusion on relevance for mammalian risk assessment
Spiroxamine - desethyl (M01) [GROUP A]	**Primary crops** Wheat Forage: 5.1% TRR; 1.11 mg/kg Straw: 2.0% TRR; 0.70 mg/kg Grain: 0.5% TRR; <0.001 mg/kg Grapes 2.1 % TRR; 0.27 mg/kg Banana Pulp: 1.1% TRR; 0.005 mg/kg Peel: 2.7% TRR; 0.18 mg/kg **Rotational crops** Leafy vegetables 12.6% TRR; 0.026 mg/kg Cereals 20.0% TRR; 0.119 mg/kg Root & tuber vegetables 9.3% TRR; 0.083 mg/kg	Not found in goat or rat. Found in laying hen (21.3% in liver, 9.3% in muscle, 8.4% in fat and 11.5% in eggs)	No data available.	Metabolite found in primary crops at <10% TRR therefore not considered relevant for risk assessment. Metabolite found >10% TRR in rotational crops but actual residue levels were very low therefore not considered relevant for risk assessment.

Plant Metabolite	Maximum levels of residue in plants	Metabolite found in animal studies?	Mammalian toxicity data available?	Conclusion on relevance for mammalian risk assessment
Spiroxamine - despropyl (M02) [GROUP A]	**Primary crops** Wheat Forage: 4.6% TRR; 0.49 mg/kg Straw: 4.2% TRR; 3.48 mg/kg Grain: 3.0% TRR; 0.002 mg/kg Grapes 1.5% TRR; 0.20 mg/kg Banana Pulp: 0.5% TRR; 0.002 mg/kg Peel: 2.9% TRR; 0.19 mg/kg **Rotational crops** Leafy vegetables 51.2% TRR; 0.053 mg/kg Cereals 46.6% TRR; 0.190 mg/kg Root & tuber vegetables 21.1% TRR; 0.188 mg/kg	Not found in goat or rat. Found in laying hen (21.7% in liver, 11.3% in muscle, 3.4% in fat and 10.2% in eggs)	No data available.	Metabolite found in primary crops at <10% TRR therefore not considered relevant for risk assessment. Metabolite found <10% TRR in rotational crops but actual residue levels were very low therefore not considered relevant for risk assessment.
Spiroxamine - N-oxide (M03) [GROUP A]	**Primary crops** Wheat Forage: 12.7% TRR; 3.06 mg/kg Straw: 22.0% TRR; 7.68 mg/kg Grain: 17.8% TRR; 0.012 mg/kg Grapes 4.3% TRR; 0.61 mg/kg Banana Pulp: 1.2% TRR; 0.007 mg/kg Peel: 4.9% TRR; 0.23 mg/kg **Rotational crops** Cereals 7.4% TRR; 0.235 mg/kg	Not found in goat or laying hen Found in rat (found in liver at low amounts of 0.6%)	Acute oral rat LD_{50} 707 mg/kg bw 28-day rat oral dietary NOAEL 12.9/13.3 mg/kg bw/day for males/females 90-day rat oral dietary NOAEL 8.8/9.7 mg/kg bw/day for males/females	Metabolite found in wheat at >10% TRR therefore considered relevant for risk assessment. Tox data are available and show that metabolite toxicity is comparable to that of spiroxamine. Metabolite found in the rat metabolism study therefore toxicity data and associated assessment for parent considered to cover this metabolite.

Plant Metabolite	Maximum levels of residue in plants	Metabolite found in animal studies?	Mammalian toxicity data available?	Conclusion on relevance for mammalian risk assessment
Spiroxamine - N-formyl-desethyl (M04) [GROUP A]	**Primary crops** Wheat Forage: 5.8% TRR; 1.40 mg/kg Straw: 9.7% TRR; 8.06 mg/kg Grain: 6.9% TRR; 0.005 mg/kg Grapes Not found Banana Not found **Rotational crops** Cereals 6.4% TRR; 0.204 mg/kg	Not found in goat, rat or laying hen	No data available.	Metabolite found in primary crops and rotational crops at <10% TRR therefore not considered relevant for risk assessment.
Spiroxamine - hydroxyl (M05) [GROUP A]	**Primary crops** Wheat Forage: 7.1% TRR; 1.71 mg/kg Straw: 5.2% TRR; 4.32 mg/kg Grain: 1.6% TRR; 0.001 mg/kg Grapes 0.3% TRR; 0.04 mg/kg Banana Not found **Rotational crops** Leafy vegetables 17.2% TRR; 0.146 mg/kg Cereals 2.5% TRR; 0.49 mg/kg Root & tuber vegetables 3.6% TRR; 0.032 mg/kg	Not found in goat, rat or laying hen	No data available.	Metabolite found in rotational crops at >10% TRR in leafy vegetables but the actual residues level is very low therefore not considered relevant for risk assessment.
Spiroxamine - hydroxy-despropyl (M09) [GROUP A]	**Primary crops** Wheat Forage: not found Straw: 0.3% TRR; 0.11 mg/kg Grain: not found Grapes Not found Banana Not found	Not found in goat, rat or laying hen	No data available.	Metabolite found in primary crops and rotational crops at <10% TRR therefore not considered relevant for risk assessment.

Plant Metabolite	Maximum levels of residue in plants	Metabolite found in animal studies?	Mammalian toxicity data available?	Conclusion on relevance for mammalian risk assessment
Spiroxamine – cyclohexanol (M13) [Group B]	**Primary crops** Not found	Not found in goat, rat or laying hen	Acute oral rat LD_{50} 4200 mg/kg bw Acute dermal rabbit LD_{50} >5000 mg/kg bw 28-day rat oral (gavage) NOAEL 50 mg/kg bw/day It was concluded that M13 is less toxic than the parent, spiroxamine in the rat with a *ca.* 9-fold, 2-fold and 8-fold increase in sub-acute, maternal and developmental NOAELs, respectively when compared to the spiroxamine equivalent studies	Metabolite not found in crop metabolism studies therefore not considered relevant for risk assessment. Tox data are available and confirm M13 to be less toxic than parent. M13 data used to represent the toxicity of all Group B metabolites.
Spiroxamine cyclohexanol acetate (M-13 acetate) [Group B]	**Primary crops** Not found	Not found in goat, rat or laying hen	Rat developmental NOAEL maternal toxicity 40 mg/kg bw/day Rat developmental NOAEL 160 mg/kg bw/day Group B metabolites considered to be covered by available data for M13 which confirm that this metabolite is less toxic than spiroxamine.	Metabolite not found in crop metabolism studies therefore not considered relevant to the risk assessment. Tox data are available and suggest lower toxicity than parent spiroxamine.

Plant Metabolite	Maximum levels of residue in plants	Metabolite found in animal studies?	Mammalian toxicity data available?	Conclusion on relevance for mammalian risk assessment
Spiroxamine-diol (M14) [Group B]	**Primary crops** Banana pulp (12.8% TRR; 0.057 mg/kg- hydrolysis product) Grapes (13.0% TRR; 0.44 mg/kg- hydrolysis product) Spring wheat straw (0.2% TRR; 0.070 mg/kg- hydrolysis product) Spring wheat grain (2.6% TRR; 0.002 mg/kg- hydrolysis product) **Rotational crops** Swiss chard leaves (8.8-13.2% TRR; 0.01 mg/kg- hydrolysis product) Wheat straw (3.5-4% TRR; 0.05 mg/kg- hydrolysis product) Turnip tops (4.4-13.0% TRR; 0.02 mg/kg- hydrolysis product)	Not found in goat, rat or laying hen	No data available.	Metabolite found in plants at >10% TRR but the actual residues level is very low therefore not considered relevant for risk assessment. Toxicity would be covered by parent assessment as M13 data confirm this group of metabolites to be less toxic than parent.
Spiroxamine-ketone (M15) [Group B]	**Primary crops** Grapes (1.3% TRR- hydrolysis product) Spring wheat straw (5.5% TRR- hydrolysis product) Spring wheat grain (4.6% TRR- hydrolysis product)	Not found in goat, rat or laying hen	No data available.	Metabolite found in plants at <10% TRR therefore not considered relevant for risk assessment.
Spiroxamine-hydroxy-ketone (M16) [Group B]	**Primary crops** Grapes (0.5% TRR- hydrolysis product) Spring wheat straw (1.0% TRR- hydrolysis product) Spring wheat grain (7.6% TRR- hydrolysis product) **Rotational crops** Swiss chard leaves (15.6-29.3% TRR; 0.04 mg/kg- hydrolysis product) Wheat straw (8.9-11.6% TRR; 0.15 mg/kg- hydrolysis product) Turnip tops (11.7-37.3% TRR; 0.17 mg/kg- hydrolysis product)	Not found in goat, rat or laying hen	No data available.	Metabolite found in plants at >10% TRR but the actual residues level is very low therefore not considered relevant for risk assessment. Toxicity would be covered by parent assessment as M13 data confirm this group of metabolites to be less toxic than parent.

Plant Metabolite	Maximum levels of residue in plants	Metabolite found in animal studies?	Mammalian toxicity data available?	Conclusion on relevance for mammalian risk assessment
Spiroxamine - hydroxy-N-oxide glucoside (M20) [Group A]	**Primary crops** Wheat Forage: 0.7% TRR; 0.08 mg/kg Straw: 2.0% TRR; 0.70 mg/kg Grain: not found Grapes Not found Banana Not found **Rotational crops** Swiss chard leaves (2.5% TRR; <0.01 mg/kg)) Wheat straw (2.1-2.6% TRR; 0.03 mg/kg) Turnip tops (8.4-10.4% TRR; 0.04 mg/kg)	Not found in goat, rat or laying hen	No data available.	Metabolite found in primary crops at <10% TRR with the exception of turnip tops but the actual residues level is very low. Therefore this metabolite is not considered relevant for risk assessment.
Spiroxamine - hydroxy-N-oxide malonyl glucoside (M21) [Group A]	**Primary crops** Wheat Forage: 2.0% TRR; 0.22 mg/kg Straw: 3.1% TRR; 2.57 mg/kg Grain: not found Grapes Not found Banana Not found **Rotational crops** Swiss chard leaves (1.6% TRR; <0.01 mg/kg) Wheat straw (4.4% TRR; 0.06 mg/kg) Turnip tops (1.7-3.7% TRR; <0.01 mg/kg)	Not found in goat, rat or laying hen	No data available.	Metabolite found in plants at <10% TRR therefore not considered relevant for risk assessment.

Plant Metabolite	Maximum levels of residue in plants	Metabolite found in animal studies?	Mammalian toxicity data available?	Conclusion on relevance for mammalian risk assessment
Spiroxamine-diol-diglycoside (M24) [Group B]	**Primary crops** Grapes (14.8% TRR – main component of metabolite group 12; 0.50 mg/kg) **Rotational crops** Swiss chard leaves (3.0% TRR; <0.01 mg/kg) Wheat straw (1.9-2.2% TRR; 0.020 mg/kg- Turnip roots (7.8% TRR; <0.01 mg/kg) Turnip tops (2.0-4.3% TRR; <0.01 mg/kg)	Not found in goat, rat or laying hen	No data available.	Metabolite found in grapes at >10% TRR but the actual residues level is very low therefore not considered relevant for risk assessment. Toxicity would be covered by parent assessment as M13 data confirm this group of metabolites to be less toxic than parent.
Spiroxamine - aminodiol (M28) [GROUP C]	**Primary crops** Wheat Not found Grapes 37.5% TRR; 4.91 mg/kg Banana Pulp: 31.2% TRR; 0.173 mg/kg Peel: 37.2% TRR; 2.45 mg/kg **Rotational crops** Leafy vegetables 3.9% TRR; 0.014 mg/kg Cereals 0.6% TRR; 0.024 mg/kg Root & tuber vegetables 4.9% TRR; 0.005 mg/kg	Found in rat at 2.2 – 5.7% of dose	Acute oral rat LD_{50} >550 <2000 mg/kg bw 28-day rat oral dietary NOAEL 28.4/31.4 mg/kg bw/day for males/females Developmental rat oral (gavage) NOAEL maternal toxicity 150 mg/kg bw/day and developmental NOAEL 30 mg/kg bw/day It was concluded that M28 is less toxic than the parent, spiroxamine in the rat with a *ca.* 15-fold, 9-fold and 2-fold increase in sub-acute, maternal and developmental NOAELs, respectively when compared to the spiroxamine equivalent studies.	Metabolite found in grapes and banana at >10% TRR therefore relevant for the risk assessment. Tox data are available and confirm that toxicity is less than parent. Furthermore, M28 was found in the rat metabolism study therefore the toxicity and the associated risk assessment is considered to be covered by the assessment of parent spiroxamine. M28 data used to represent the toxicity of all Group C metabolites.

Plant Metabolite	Maximum levels of residue in plants	Metabolite found in animal studies?	Mammalian toxicity data available?	Conclusion on relevance for mammalian risk assessment
Spiroxamine - aminodiol-N-oxide (M29) [GROUP C]	**Primary crops** Wheat Not found Grapes 0.1% TRR; 0.01 mg/kg Banana Not found **Rotational crops** Leafy vegetables 5.2% TRR; 0.021 mg/kg Root & tuber vegetables 4.8% TRR; 0.005 mg/kg	Not found in goat, rat or laying hen	No data available. Group C metabolites considered to be covered by available data for M28 which confirm that this metabolite is less toxic than spiroxamine.	Metabolite found in primary crops and rotational crops at <10% TRR therefore not considered relevant for risk assessment. Toxicity would be covered by parent assessment as M28 data confirm this group of metabolites to be less toxic than parent.
Spiroxamine - desethyl-aminodiol (M30) [GROUP C]	**Primary crops** Wheat Not found Grapes 1.1% TRR; 0.14 mg/kg Banana Pulp: 0.6% TRR; 0.003 mg/kg Peel: 0.9% TRR; 0.06 mg/kg	Not found in goat, rat or laying hen	No data available. Group C metabolites considered to be covered by available data for M28 which confirm that this metabolite is less toxic than spiroxamine.	Metabolite found in primary crops at <10% TRR therefore not considered relevant for risk assessment. Toxicity would be covered by parent assessment as M28 data confirm this group of metabolites to be less toxic than parent.
Spiroxamine - despropyl-aminodiol (M31) [GROUP C]	**Primary crops** Wheat Not found Grapes 1.2% TRR; 0.16 mg/kg Banana Pulp: 0.6% TRR; 0.003 mg/kg Peel: 0.9% TRR; 0.06 mg/kg **Rotational crops** Cereals 1.7% TRR; 0.034 mg/kg Root & tuber vegetables 6.1% TRR; 0.006 mg/kg	Not found in goat, rat or laying hen	No data available. Group C metabolites considered to be covered by available data for M28 which confirm that this metabolite is less toxic than spiroxamine.	Metabolite found in primary crops and rotational crops at <10% TRR therefore not considered relevant for risk assessment. Toxicity would be covered by parent assessment as M28 data confirm this group of metabolites to be less toxic than parent.

Plant Metabolite	Maximum levels of residue in plants	Metabolite found in animal studies?	Mammalian toxicity data available?	Conclusion on relevance for mammalian risk assessment
Spiroxamine-cyclohexanol-glucopyranosyl-pentose (M33) [GROUP B]	**Primary crops** Grapes (19.1% TRR; 0.650 mg/kg)	Not found in goat, rat or laying hen	No data available.	Metabolite found in plants at >10% TRR but the actual residues level is very low therefore not considered relevant for risk assessment. Toxicity would be covered by parent assessment as M13 data confirm this group of metabolites to be less toxic than parent.
Spiroxamine-cyclohexanol-glucopyranosyl-glucopyranosyl-pentose (M34) [GROUP B]	**Primary crops** Grapes (3.5% TRR; 0.130 mg/kg)	Not found in goat, rat or laying hen	No data available.	Metabolite found in plants at <10% TRR therefore not considered relevant for risk assessment.
Spiroxamine docosanoic acid ester (M35) [GROUP B]	**Primary crops** <u>Wheat</u> Not found <u>Grapes</u> 13.0% TRR; 0.44 mg/kg <u>Banana</u> Not found	Not found in goat, rat or laying hen	No data on M35. Group B metabolites considered to be covered by available data for M13 which confirm that this metabolite is less toxic than spiroxamine.	Metabolite found in grapes at >10% TRR. Available data with M13 used to cover this Group B metabolite and confirms that the toxicity is lower than that of the parent. Thus, the risk to M35 is considered to be covered by the assessment for parent.

Plant Metabolite	Maximum levels of residue in plants	Metabolite found in animal studies?	Mammalian toxicity data available?	Conclusion on relevance for mammalian risk assessment
Spiroxamine tetracosanoic acid ester (M36) [GROUP B]	**Primary crops** Wheat Not found Grapes 4.2% TRR; 0.14 mg/kg Banana Not found	Not found in goat, rat or laying hen	No data on M36. Group B metabolites considered to be covered by available data for M13 which confirm that this metabolite is less toxic than spiroxamine.	Metabolite found in primary crops at <10% TRR therefore not considered relevant for risk assessment. Toxicity would be covered by parent assessment as M13 data confirm this group of metabolites to be less toxic than parent.
Spiroxamine-cyclohexenol (M37) [GROUP B]	**Primary crops** Grapes (3.2% TRR; 0.11 mg/kg-hydrolysis product)	Not found in goat, rat or laying hen	No data available.	Metabolite found in plants at <10% TRR therefore not considered relevant for risk assessment.
Spiroxamine – N-formyl-despropyl (M38) [GROUP A]	**Primary crops** Not found **Rotational crops** Cereals 7.6% TRR; 0.243 mg/kg	Not found in goat, rat or laying hen	No data available.	Metabolite found in rotational crops only and at <10% TRR therefore not considered relevant for risk assessment.
Spiroxamine – hydroxy-despropyl glycoside (M39) [GROUP A]	**Rotational crops** Leafy vegetables 2.8% TRR; 0.019 mg/kg Cereals 5.9% TRR; 0.232 mg/kg Root & tuber vegetables 21.3% TRR; 0.063 mg/kg	Not found in goat, rat or laying hen	No data available.	Metabolite found in rotational crops at >10% TRR in root and tuber vegetables but the actual residues level is very low therefore not considered relevant for risk assessment.
Spiroxamine – hydroxy glycoside (M40) [GROUP A]	**Primary crops** Not found **Rotational crops** Leafy vegetables 4.7% TRR; 0.040 mg/kg Cereals 2.9% TRR; 0.088 mg/kg Root & tuber vegetables 7.6% TRR; 0.068 mg/kg	Not found in goat, rat or laying hen	No data available.	Metabolite found in rotational crops only and at <10% TRR therefore not considered relevant for risk assessment.

Plant Metabolite	Maximum levels of residue in plants	Metabolite found in animal studies?	Mammalian toxicity data available?	Conclusion on relevance for mammalian risk assessment
Spiroxamine – hydroxy-desethyl glycoside (M42) [GROUP A]	**Primary crops** Not found **Rotational crops** Leafy vegetables 1.6% TRR; 0.005 mg/kg Cereals 6.5% TRR; 0.129 mg/kg Root & tuber vegetables 14.6% TRR; 0.044 mg/kg	Not found in goat, rat or laying hen	No data available.	Metabolite found in rotational crops at >10% TRR in root and tuber vegetables but the actual residues level is very low therefore not considered relevant for risk assessment.
Spiroxamine – desethyl acid glycoside (M43) [GROUP A]	**Primary crops** Not found **Rotational crops** Leafy vegetables 1.8% TRR; 0.015 mg/kg Cereals 3.4% TRR; 0.088 mg/kg Root & tuber vegetables 5.7% TRR; 0.051 mg/kg	Not found in goat, rat or laying hen	No data available.	Metabolite found in rotational crops only and at <10% TRR therefore not considered relevant for risk assessment.
Spiroxamine – acid glycoside (M44) [GROUP A]	**Primary crops** Not found **Rotational crops** Leafy vegetables 4.6% TRR; 0.019 mg/kg Cereals 6.4% TRR; 0.126 mg/kg Root & tuber vegetables 11.6% TRR; 0.027 mg/kg	Not found in goat, rat or laying hen	No data available.	Metabolite found in rotational crops at >10% TRR in root and tuber vegetables but the actual residues level is very low therefore not considered relevant for risk assessment.
Spiroxamine – despropyl acid glycoside (M45) [GROUP A]	**Primary crops** Not found **Rotational crops** Leafy vegetables 5.5% TRR; 0.019 mg/kg Cereals 3.7% TRR; 0.145 mg/kg Root & tuber vegetables 9.1% TRR; 0.002 mg/kg	Not found in goat, rat or laying hen	No data available.	Metabolite found in rotational crops only and at <10% TRR therefore not considered relevant for risk assessment.

M01 and M02 were found in the rotational crop studies at >10% TRR however the absolute residue values were very low therefore these metabolites are not considered to be relevant to the mammalian risk assessment.

M03 was found in wheat at >10% TRR. Toxicology data for M03 demonstrate that this metabolite is of similar or lower toxicity than spiroxamine. Furthermore, this metabolite was found in the rat metabolism study. It is therefore considered that the risk assessment of parent spiroxamine covers the risk from exposure to this metabolite.

M28 was found in grapes and banana at >10% TRR. Toxicology data are available for M28 and confirm that this metabolite is 15-fold, 9-fold and 2-fold less toxic in sub-acute, maternal and developmental parameters than spiroxamine. Furthermore, this metabolite was found in the rat metabolism study. Therefore the risk from exposure to this metabolite is considered to be covered by the assessment for parent.

M35 was also found in grapes at >10%TRR. The toxicology data generated for M28 are considered to also cover this metabolite and confirm that the metabolite is less toxic than parent. Thus, the risk from exposure to this plant metabolite is considered to be covered by the assessment for parent.

M04, M05, M09, M14, M15, M16, M20, M21, M24, M29, M30, M31, M33, M34, M36, M37, M38, M39, M40, M42, M43, M44 and M45 were found in the crop metabolism studies at either <10% TRR or very low absolute amounts and were therefore not considered to be relevant for risk assessment. Specific dietary risk assessment for these plant metabolites of spiroxamine is therefore not considered to be necessary.

A detailed consideration of the metabolites of prothioconazole is not considered to be an integral part of the Renewal of Approval of spiroxamine. Thus, prothioconazole data have been used in the risk assessment of Prothioconazole + Spiroxamine EC 460 but the information has been taken from the 2007 EFSA Conclusion for prothioconazole. Prothioconazole-desthio is a metabolite of prothioconazole that occurred in amounts of >10% of the TRR on plant material and has been included in the residue definition. Therefore, a risk assessment is conducted. Furthermore, mammalian toxicity data are available. To facilitate the risk assessment for birds and mammals, a 100 % conversion of the parent compound prothioconazole into the metabolite prothioconazole-desthio has been assumed.

Dietary risk assessment for mammals

Exposure

The proposed use of Prothioconazole + Spiroxamine EC 460 is for either one or two applications (14-day interval) to cereals (BBCH 20 - 69) at a maximum application rate of 1.25 L product/ha (equivalent to 375 g spiroxamine/ha and 200 g prothioconazole/ha). Risk assessments for both one and two applications at this rate have been conducted and are considered to cover all other proposed uses of this product.

Isomers

The risk assessments for birds & mammals involves potential chronic exposure of these organisms to residues in plants therefore it may be necessary to apply an uncertainty factor (UF) to the chronic avian and mammalian risk assessments. The acute risk assessment need not have an UF applied as exposure in this scenario is immediate. However, the chronic risk assessment considers exposure over a prolonged period therefore potential changes in isomeric ratio needs to be considered. For the bird and mammal risk assessments the same approach taken in the residues section for the consumer risk assessment, with respect to isomers, has been followed. Based on the current residues data set for spiroxamine, there are no indications of a significant change in isomer ratios therefore no additional factor need be applied to the risk assessments below (*i.e.* an UF of 1.0 has been used).

Risk assessment

The risk assessments have been conducted in accordance with the EFSA (2009) Bird & Mammal Guidance document. Each assessment starts with a screening step followed by a Tier I assessment if required. Finally, refined risk assessments have been presented where required.

The acute 'daily dietary dose' (DDD) is calculated by multiplying the shortcut value (SV) based on the 90th percentile residues by the application rate in kg a.s./ha.

DDD_A = application rate (kg a.s./ha) x SV_{90}

The long-term 'daily dietary dose' (DDD) is calculated by multiplying the SV based on the mean residues by the application rate in kg a.s./ha and (only for the long-term risk assessment) a time weighted

average residue exposure (f_{twa}). The f_{twa} based upon a default DT_{50} of 10 days is 0.53, as given in EFSA guidance (2009).

DDD_{LT} = application rate (kg a.s./ha) x SV_m x f_{twa}

Acute risk is assessed by comparing the relevant daily dietary dose (DDD) values with the appropriate LD_{50} endpoint to give an acute Toxicity: Exposure Ratio (TER_A):

$$TER_A = \frac{LD_{50}\ (\text{mg/kg bw})}{\text{DDD}}$$

TER_A values which exceed a trigger value of 10 indicate an acceptable acute risk.

Derivation of the short-term toxicity exposure ratio is no longer a requirement according to the EFSA Guidance Document (2009) so a short-term risk assessment has not been presented.

Long-term risk is assessed by comparing the long-term DDD values with the worst case NOAEL/NOEL from the reproduction studies, expressed as daily dietary dose, to give a long-term toxicity exposure ratio (TER_{LT}):

$$TER_{LT} = \frac{NOAEL\ (\text{mg/kg bw/day})}{\text{DDD}\ (\text{mg/kg bw/day})}$$

TER_{LT} values which exceed a trigger value of 5 indicate acceptable chronic risk.

1 x 1.25 L/ha application of Prothioconazole & Spiroxamine EC 460

The screening step assessments for the acute and reproductive risks are presented below for spiroxamine, prothioconazole and prothioconazole-desthio.

Table CP 10.1.2-5 Screening step assessment for acute and long-term/reproductive risk to mammals for the proposed use of Prothioconazole + Spiroxamine 460 in cereals - Spiroxamine

Intended use	Cereals				
Active substance/product	Spiroxamine / Prothioconazole + Spiroxamine 460				
Application rate (g a.s./ha)	1 × 375				
Acute toxicity (mg a.s./kg bw)	460				
TER criterion	10				
Crop scenario	Indicator species	SV_{90}	MAF_{90}	DDD_{90} (mg a.s./kg bw/d)	TER_A
Cereals	Small herbivorous mammal	118.4	1.0	44.4	10.4
Reprod. toxicity (mg/kg bw/d)	21.0				
TER criterion	5				
Crop scenario	Indicator species	SV_m	MAF_m × TWA	DDD_m (mg a.s./kg bw/d)	TER_{LT}
Cereals	Small herbivorous mammal	48.3	1.0 x 0.53	9.60	**2.19**

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity exposure ratio. TER values shown in bold fall below the relevant trigger

For the proposed use of Prothioconazole + Spiroxamine EC 460 on cereals (1 x 1.25 L product/ha at BBCH 30 - 69), the acute risks to mammals from dietary exposure to spiroxamine are acceptable (TER ≥10) and no further acute risk assessment is necessary. Potential reproductive risks to mammals from

dietary exposure to spiroxamine have been identified (TER <5) therefore a Tier I reproductive risk assessment has been conducted and presented below.

Table CP 10.1.2-6 Screening step assessment for acute and long-term/reproductive risk to mammals for the proposed use of Prothioconazole + Spiroxamine 460 in cereals - Prothioconazole

Intended use	Cereals				
Active substance/product	Prothioconazole / Prothioconazole + Spiroxamine 460				
Application rate (g a.s./ha)	1 × 200				
Acute toxicity (mg a.s./kg bw)	>6200				
TER criterion	10				
Crop scenario	Indicator species	SV_{90}	MAF_{90}	DDD_{90} (mg a.s./kg bw/d)	TER_A
Cereals	Small herbivorous mammal	118.4	1.0	23.7	262
Reprod. toxicity (mg a.s./kg bw/d)	95.6				
TER criterion	5				
Crop scenario	Indicator species	SV_m	MAF_m × TWA	DDD_m (mg a.s./kg bw/d)	TER_{LT}
Cereals	Small herbivorous mammal	48.3	1.0 x 0.53	5.12	18.7

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity exposure ratio.

For the proposed use of Prothioconazole + Spiroxamine EC 460 on cereals (1 x 1.25 L product/ha at BBCH 30 - 69) the acute and reproductive risks to mammals from dietary exposure to prothioconazole are considered to be acceptable (TER ≥10 and ≥5 for acute and reproductive risks, respectively). No further risk assessment for prothioconazole exposure is necessary.

Table CP 10.1.2-7 Screening step assessment for acute and long-term/reproductive risk to mammals for the proposed use of Prothioconazole + Spiroxamine 460 in cereals – Prothioconazole-desthio

Intended use	Cereals				
Active substance/product	Prothioconazole-desthio / Prothioconazole + Spiroxamine 460				
Application rate (g a.s./ha)	1 × 200				
Acute toxicity (mg a.s./kg bw)	2235				
TER criterion	10				
Crop scenario	Indicator species	SV_{90}	MAF_{90}	DDD_{90} (mg a.s./kg bw/d)	TER_A
Cereals	Small herbivorous mammal	118.4	1.0	23.7	94.4
Reprod. toxicity (mg a.s./kg bw/d)	10				
TER criterion	5				
Crop scenario	Indicator species	SV_m	MAF_m × TWA	DDD_m (mg a.s./kg bw/d)	TER_{LT}
Cereals	Small herbivorous mammal	48.3	1.0 x 0.53	5.12	**1.95**

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity exposure ratio. TER values shown in bold fall below the relevant trigger

For the proposed use of Prothioconazole + Spiroxamine EC 460 on cereals (1 x 1.25 L product/ha at BBCH 30 - 69), the acute risks to mammals from dietary exposure to prothioconazole-desthio are acceptable (TER ≥100 and no further acute risk assessment is necessary. Potential reproductive risks to mammals from dietary exposure to prothioconazole-desthio have been identified (TER <5) therefore a Tier I reproductive risk assessment has been conducted and presented below.

Reproductive risk assessment (Tier I)

Table CP 10.1.2-8 Tier I assessment for reproductive risk to mammals for the proposed use of Prothioconazole + Spiroxamine 460 in cereals – Spiroxamine

Intended use	Cereals				
Active substance/product	Spiroxamine / Prothioconazole + Spiroxamine 460				
Application rate (g a.s./ha)	1 × 375				
Reprod. toxicity (mg a.s./kg bw/d)	21.0				
TER criterion	5				
Crop scenario Growth stage	Generic focal species	SV_m	MAF_m × TWA	DDD_m (mg a.s./kg bw/d)	TER_{LT}
Cereals BBCH >20	Small insectivorous mammal "shrew"	1.9	1.0 x 0.53	0.378	55.6
Cereals BBCH 30-39	Small omnivorous mammal "mouse"	3.9	1.0 x 0.53	0.775	27.1
Cereals BBCH >40	Small herbivorous mammal "vole"	21.7	1.0 x 0.53	4.31	**4.87**
Cereals BBCH >40	Small herbivorous mammal "mouse"	2.3	1.0 x 0.53	0.457	45.9

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger

For the proposed use of Prothioconazole + Spiroxamine EC 460 on cereals (1 x 1.25 L product/ha at BBCH 30 - 69) the reproductive risks to mammals from dietary exposure to spiroxamine are considered to be acceptable (TER≥5) for all relevant scenarios with the exception of the small herbivorous mammal "vole" scenario. A refined risk assessment for this scenario has been presented below.

Table CP 10.1.2-9 Tier I assessment for reproductive risk to mammals for the proposed use of Prothioconazole + Spiroxamine 460 in cereals – Prothioconazole-desthio

Intended use	Cereals				
Active substance/product	Prothioconazole-desthio / Prothioconazole + Spiroxamine 460				
Application rate (g a.s./ha)	1 × 200				
Reprod. toxicity (mg a.s./kg bw/d)	10				
TER criterion	5				
Crop scenario Growth stage	Generic focal species	SV_m	MAF_m < TWA	DDD_m (mg a.s./kg bw/d)	TER_{LT}
Cereals BBCH >20	Small insectivorous mammal "shrew"	1.9	1.0 x 0.53	0.201	49.7
Cereals BBCH 30-39	Small omnivorous mammal "mouse"	3.9	1.0 x 0.53	0.413	24.2
Cereals BBCH >40	Small herbivorous mammal "vole"	21.7	1.0 x 0.53	2.30	**4.35**
Cereals BBCH >40	Small herbivorous mammal "mouse"	2.3	1.0 x 0.53	0.244	41.0

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger

For the proposed use of Prothioconazole + Spiroxamine EC 460 on cereals (1 x 1.25 L product/ha at BBCH 30 - 69) the reproductive risks to mammals from dietary exposure to prothioconazole-desthio are considered to be acceptable (TER ≥5) for all relevant scenarios with the exception of the small herbivorous mammal "vole" scenario. A refined risk assessment for this scenario has been presented below.

2 x 1.25 L/ha application of Prothioconazole & Spiroxamine EC 460

The screening step assessments for the acute and reproductive risks are presented below for spiroxamine, prothioconazole and prothioconazole-desthio.

Table CP 10.1.2-10 Screening step assessment for acute and long-term/reproductive risk to mammals for the proposed use of Prothioconazole + Spiroxamine 460 in cereals - Spiroxamine

Intended use	Cereals				
Active substance/product	Spiroxamine / Prothioconazole + Spiroxamine 460				
Application rate (g a.s./ha)	2 × 375				
Acute toxicity (mg a.s./kg bw)	460				
TER criterion	10				
Crop scenario	Indicator species	SV_{90}	MAF_{90}	DDD_{90} (mg a.s./kg bw/d)	TER_A
Cereals	Small herbivorous mammal	118.4	1.2	53.3	**8.63**
Reprod. toxicity (mg/kg bw/d)	21.0				
TER criterion	5				
Crop scenario Growth stage	Indicator species	SV_m	MAF_m × TWA	DDD_m (mg a.s./kg bw/d)	TER_{LT}
Cereals	Small herbivorous mammal	48.3	1.4 x 0.53	18.4	**1.56**

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity exposure ratio. TER values shown in bold fall below the relevant trigger.

For the proposed use of Prothioconazole + Spiroxamine EC 460 on cereals (2 x 1.25 L product/ha at BBCH 30 - 69), potential acute and reproductive risks to mammals from dietary exposure to spiroxamine have been identified (TER < 10 and < 5, respectively) therefore a Tier I reproductive risk assessment has been conducted and presented below.

Table CP 10.1.2-11 Screening step assessment for acute and long-term/reproductive risk to mammals for the proposed use of Prothioconazole + Spiroxamine 460 in cereals - Prothioconazole

Intended use	Cereals				
Active substance/product	Prothioconazole / Prothioconazole + Spiroxamine 460				
Application rate (g a.s./ha)	2 × 200				
Acute toxicity (mg a.s./kg bw)	6200				
TER criterion	10				
Crop scenario	Indicator species	SV_{90}	MAF_{90}	DDD_{90} (mg a.s./kg bw/d)	TER_A
Cereals	Small herbivorous mammal	118.4	1.2	28.4	218
Reprod. toxicity (mg/kg bw/d)	95.6				
TER criterion	5				
Crop scenario Growth stage	Indicator species	SV_m	MAF_m × TWA	DDD_m (mg a.s./kg bw/d)	TER_{LT}
Cereals	Small herbivorous mammal	48.3	1.4 x 0.53	7.17	13.3

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity exposure ratio.

For the proposed use of Prothioconazole + Spiroxamine EC 460 on cereals (2 x 1.25 L product/ha at BBCH 30 - 69) the acute and reproductive risks to mammals from dietary exposure to prothioconazole

are considered to be acceptable (TER ≥10 and ≥5 for acute and reproductive risks, respectively). No further risk assessment for prothioconazole exposure is necessary.

Table CP 10.1.2-12 Screening step assessment for acute and long-term/reproductive risk to mammals for the proposed use of Prothioconazole + Spiroxamine 460 in cereals – Prothioconazole-desthio

Intended use	Cereals				
Active substance/product	Prothioconazole-desthio / Prothioconazole + Spiroxamine 460				
Application rate (g a.s./ha)	2 × 200				
Acute toxicity (mg a.s./kg bw)	2235				
TER criterion	10				
Crop scenario	Indicator species	SV_{90}	MAF_{90}	DDD_{90} (mg a.s./kg bw/d)	TER_A
Cereals	Small herbivorous mammal	118.4	1.2	28.4	78.7
Reprod. toxicity (mg/kg bw/d)	10				
TER criterion	5				
Crop scenario	Indicator species	SV_m	MAF_m × TWA	DDD_m (mg a.s./kg bw/d)	TER_{LT}
Cereals	Small herbivorous mammal	48.3	1.4 x 0.53	7.17	**1.40**

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity exposure ratio. TER values shown in bold fall below the relevant trigger.

For the proposed use of Prothioconazole + Spiroxamine EC 460 on cereals (2 x 1.25 L product/ha at BBCH 30 - 69), the acute risks to mammals from dietary exposure to prothioconazole-desthio are acceptable (TER ≥10) and no further acute risk assessment is necessary. Potential reproductive risks to mammals from dietary exposure to prothioconazole-desthio have been identified (TER <5) therefore a Tier I reproductive risk assessment has been conducted and presented below.

Acute risk assessment (Tier I)

Table CP 10.1.2-13 Tier I assessment for acute risk to mammals for the proposed use of Prothioconazole + Spiroxamine 460 in cereals – Spiroxamine

Intended use	Cereals				
Active substance/product	Spiroxamine / Prothioconazole + Spiroxamine 460				
Application rate (g a.s./ha)	2 × 375				
Acute toxicity (mg a.s./kg bw)	460				
TER criterion	10				
Crop scenario Growth stage	Generic focal species	SV_{90}	MAF_{90}	DDD_{90} (mg a.s./kg bw/d)	TER_A
Cereals BBCH <20	Small insectivorous mammal "shrew"	5.4	1.2	2.43	189
Cereals BBCH 30-39	Small omnivorous mammal "mouse"	8.6	1.2	3.87	119
Cereals BBCH >40	Small herbivorous mammal "vole"	40.9	1.2	18.4	25.0

Intended use	Cereals
Active substance/product	Spiroxamine / Prothioconazole + Spiroxamine 460
Application rate (g a.s./ha)	2 × 375
Acute toxicity (mg a.s./kg bw)	460
TER criterion	10

Crop scenario Growth stage	Generic focal species	SV_{90}	MAF_{90}	DDD_{90} (mg a.s./kg bw/d)	TER_A
Cereals BBCH >40	Small herbivorous mammal "mouse"	5.2	1.2	2.34	197

SV: shortcut value; MAF: multiple application factor; DDD: daily dietary dose; TER: toxicity to exposure ratio

For the proposed use of Prothioconazole + Spiroxamine EC 460 on cereals (2 x 1.25 L product/ha at BBCH 30 - 69) the acute risks to mammals from dietary exposure to spiroxamine are considered to be acceptable (TER ≥5) for all relevant scenarios. No further acute risk assessment is necessary.

Reproductive risk assessment (Tier I)

Table CP 10.1.2-14 Tier I assessment for reproductive risk to mammals for the proposed use of Prothioconazole + Spiroxamine 460 in cereals – Spiroxamine

Intended use	Cereals
Active substance/product	Spiroxamine / Prothioconazole + Spiroxamine 460
Application rate (g a.s./ha)	2 × 375
Reprod. toxicity (mg a.s./kg bw/d)	21.0
TER criterion	5

Crop scenario Growth stage	Generic focal species	SV_m	MAF_m × TWA	DDD_m (mg a.s./kg bw/d)	TER_{LT}
Cereals BBCH >20	Small insectivorous mammal "shrew"	1.9	1.4 x 0.53	0.529	39.7
Cereals BBCH 30-39	Small omnivorous mammal "mouse"	3.9	1.4 x 0.53	1.09	19.4
Cereals BBCH >40	Small herbivorous mammal "vole"	21.7	1.4 x 0.53	6.04	**3.48**
Cereals BBCH >40	Small herbivorous mammal "mouse"	2.3	1.4 x 0.53	0.640	32.8

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger

For the proposed use of Prothioconazole + Spiroxamine EC 460 on cereals (2 x 1.25 L product/ha at BBCH 30 - 69) the reproductive risks to mammals from dietary exposure to spiroxamine are considered to be acceptable (TER ≥5) for all relevant scenarios with the exception of the small herbivorous mammal "vole" scenario. A refined risk assessment for this scenario has been presented below.

Table CP 10.1.2-15 Tier I assessment for reproductive risk to mammals for the proposed use of Prothioconazole + Spiroxamine 460 in cereals – Prothioconazole-desthio

Intended use	Cereals
Active substance/product	Prothioconazole-desthio / Prothioconazole + Spiroxamine 460
Application rate (g a.s./ha)	2 × 200
Reprod. toxicity (mg a.s./kg bw/d)	10
TER criterion	5

Crop scenario Growth stage	Generic focal species	SV_m	$MAF_m \times TWA$	DDD_m (mg a.s./kg bw/d)	TER_{LT}
Cereals BBCH >20	Small insectivorous mammal “shrew”	1.9	1.4 x 0.53	0.282	35.5
Cereals BBCH 30-39	Small omnivorous mammal “mouse”	3.9	1.4 x 0.53	0.579	17.3
Cereals BBCH >40	Small herbivorous mammal “vole”	21.7	1.4 x 0.53	3.22	**3.11**
Cereals BBCH >40	Small herbivorous mammal “mouse”	2.3	1.4 x 0.53	0.341	29.3

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

For the proposed use of Prothioconazole + Spiroxamine EC 460 on cereals (2 x 1.25 L product/ha at BBCH 30 - 69) the reproductive risks to mammals from dietary exposure to prothioconazole-desthio are considered to be acceptable (TER ≥5) for all relevant scenarios with the exception of the small herbivorous mammal “vole” scenario. A refined risk assessment for this scenario has been presented below.

Refined reproductive risk assessment for the small herbivorous mammal “vole” scenario (BBCH >40) from exposure to spiroxamine following application of Prothioconazole + Spiroxamine EC 460

For the refined risk assessment of the small herbivorous mammal scenario, the vole has been used as the focal species. This is the generic focal species in EFSA (2009) but is also supported by study M-269779-01-1 in which small mammal species were monitored within and around winter cereal fields in Germany. The observations confirmed that wood mice and common voles were the most relevant species and most likely to be captured in cereal fields during spring and summer. Thus, the common vole is considered to be a suitable focal species for the refined risk assessment of small herbivorous mammals. Full details of the study can be found in the study summary.

In the same study PT values were determined for wood mice and common voles. For the common vole a mean PT of 0.635 was determined for cereals at principal growth stages 3 – 6. However, it is noted that the 90th percentile PT value was 1.0 therefore a PT of 1.0 has been used in the refined risk assessments below.

The EFSA (2009) diet for the vole of 100% grass/cereal shoots has also been used in the refined risk assessments below.

Several residues decline studies are available in which the residues of spiroxamine have been determined at frequent intervals following application of spiroxamine containing formulations to cereals. Report M-759383-01-1 summarises the kinetic analyses of all 24 available trials which have been conducted as part of five separate studies (M-301585-01-1, M-574326-01-1, M-578235-01-1, M-628347-02-1 and M-684671-01-1) covering wheat and barley plants in both Northern and Southern Europe. The

individual validity of each study has been discussed in the evaluation section after each study summary but all of the trials used in this assessment were considered to be valid and suitable for use in the derivation of an overall crop dissipation half-life (DT_{50}) value. It was found that spiroxamine residues dissipated relatively quickly on cereals with an overall geomean DT_{50} of 3.03 days determined. A geomean DT_{50} value of 2.74 days was determined for Northern EU and a DT_{50} of 3.83 days for Southern EU. The overall geomean DT_{50} of 3.03 days has been applied to the refined risk assessment below and is considered to be suitably representative of the decline of spiroxamine residues throughout Europe. Note that the refined DT_{50} only applies to cereals but as the vole diet is 100% grass/cereal shoots this refined value applies to the entire diet. The DT_{50} of 3.03 days has been used in place of the default value of 10 days to refine the f_{twa} value from 0.53 to 0.206 for the single application. For multiple applications a MAF x TWA value of 0.377 has been determined using a moving time window approach.

The refined risk assessments are presented below for the single application as well as for two applications of Prothioconazole + Spiroxamine EC 460 at 1.25 L product/ha.

Table CP 10.1.2-16 Refinement of the small herbivorous mammal vole scenarios for the proposed use of Prothioconazole + Spiroxamine 460 in cereals (1 x 1.25 L product/ha) – Spiroxamine - Vole

Application rate	Food type	FIR/bw [a)]	RUD [b)]	MAF [c)]	f_{twa} [d)]	PT [e)]	Dep. factor [f)]	DDD [mg a.s./kg b.w./d]	TER [g)]
1 x 0.375 kg a.s./ha	Cereals and grass	1.33	54.2	1.0	0.206	1.0	0.3	1.67	12.6

a) Default value from EFSA (2009) Appendix A
b) Default RUD values from EFSA (2009) Appendix F
c) Default MAF value of 1.0 used
d) A refined TWA value calculated using a DT_{50} value of 3.03 days
e) 90th percentile PT value from focal species study
f) Deposition value based on 70% crop interception for cereals at principal growth stages ≥4 (Appendix A: EFSA, 2009)
g) TER calculated based on reproductive endpoint of 21.0 mg a.s./kg bw/day

Table CP 10.1.2-17 Refinement of the small herbivorous mammal vole scenarios for the proposed use of Prothioconazole + Spiroxamine 460 in cereals (2 x 1.25 L product/ha) – Spiroxamine - Vole

Application rate	Food type	FIR/bw [a)]	RUD [b)]	MAF	f_{twa}	PT [d)]	Dep. factor [e)]	DDD [mg a.s./kg b.w./d]	TER [f)]
2 x 0.375 kg a.s./ha	Cereals and grass	1.33	54.2	0.377 [c)]		1.0	0.3	3.06	6.86

a) Default value from EFSA (2009) Appendix A
b) Default RUD values from EFSA (2009) Appendix F
c) A MAF×TWA value calculated using a moving time window and a DT_{50} value 3.03 days
d) 90th percentile PT value from focal species study
e) Deposition value based on 70% crop interception for cereals at principal growth stages ≥4 (Appendix A: EFSA, 2009)
f) TER calculated based on reproductive endpoint of 21.0 mg a.s./kg bw/day

For the proposed uses of Prothioconazole + Spiroxamine EC 460 on cereals (1 x 1.25 L product/ha and 2 x 1.25 L product/ha at BBCH 30 - 69) the reproductive risks to small herbivorous mammals from dietary exposure to spiroxamine are considered to be acceptable (TER ≥5).

Refined reproductive risk assessment for the small herbivorous mammal "vole" scenario (BBCH >40) from exposure to <u>prothioconazole-desthio</u> following application of Prothioconazole + Spiroxamine EC 460

For the refined risk assessment of the small herbivorous mammal scenario, the vole has been used as the focal species. As discussed above, this is the generic focal species in EFSA (2009) but is also

supported by study M-269779-01-1. A PT of 1.0 has been used in the refined risk assessments below along with the EFSA (2009) diet for the vole of 100% grass/cereal shoots.

The DT_{50} of Prothioconazole-desthio on wheat was determined to be 3.15 days (as presented in the Spiroxamine dRAR Volume 3, Annex B.9). The DT_{50} of 3.15 days has been used in place of the default value of 10 days to refine the f_{twa} value from 0.53 to 0.214 for the single application. For multiple applications a MAF x TWA value of 0.389 has been determined using a moving time window approach.

The refined risk assessments are presented below for the single application as well as for two applications of Prothioconazole + Spiroxamine EC 460 at 1.25 L product/ha.

Table CP 10.1.2-18 Refinement of the small herbivorous mammal vole scenarios for the proposed use of Prothioconazole + Spiroxamine 460 in cereals (1 x 1.25 L product/ha) – Prothioconazole-desthio - Vole

Application rate	Food type	FIR/bw [a]	RUD [b]	MAF [c]	f_{twa} [d]	PT [e]	Dep. factor [f]	DDD [mg a.s./kg b.w./d]	TER [g]
1 x 0.200 kg a.s./ha	Cereals and grass	1.33	54.2	1.0	0.214	1.0	0.3	0.926	10.8

[a] Default value from EFSA (2009) Appendix A
[b] Default RUD values from EFSA (2009) Appendix F
[c] Default MAF value of 1.0 used
[d] A refined TWA value calculated using a DT_{50} value of 3.15 days
[e] 90th percentile PT value from focal species study
[f] Deposition value based on 70% crop interception for cereals at principal growth stages ≥4 (Appendix A: EFSA, 2009)
[g] TER calculated based on reproductive endpoint of 10 mg a.s./kg bw/day

Table CP 10.1.2-19 Refinement of the small herbivorous mammal vole scenarios for the proposed use of Prothioconazole + Spiroxamine 460 in cereals (2 x 1.25 L product/ha) – Prothioconazole-desthio - Vole

Application rate	Food type	FIR/bw [a]	RUD [b]	MAF	f_{twa}	PT [d]	Dep. factor [e]	DDD [mg a.s./kg b.w./d]	TER [f]
2 x 0.200 kg a.s./ha	Cereals and grass	1.33	54.2	0.389 [c]		1.0	0.3	1.68	5.95

[a] Default value from EFSA (2009) Appendix A
[b] Default RUD values from EFSA (2009) Appendix F
[c] A MAF×TWA value calculated using a moving time window and a DT_{50} value 3.15 days
[d] 90th percentile PT value from focal species study
[e] Deposition value based on 70% crop interception for cereals at principal growth stages ≥4 (Appendix A: EFSA, 2009)
[f] TER calculated based on reproductive endpoint of 10 mg a.s./kg bw/day

For the proposed use of Prothioconazole + Spiroxamine EC 460 on cereals (1 x 1.25 L product/ha and 2 x 1.25 L product/ha at BBCH 30 - 69) the reproductive risks to small herbivorous mammals from dietary exposure to Prothioconazole-desthio are considered to be acceptable (TER ≥5).

Alternative refinement of the small herbivorous mammal "vole" scenario

The relevance of the vole in agricultural areas has frequently been discussed and its use in risk assessment questioned. In the literature paper M-476622-01-1 it is stated that the preferred primary habitat of common voles is steppe, which comprises grassland, pasture and meadow with mixed grassland, herbs and weeds that provide appropriate cover to avoid predation. It is also stated that although the common vole is indicated as the representative generic focal species in screening and tier 1 risk assessments under EFSA (2009), population dynamics and habitat preferences indicate that in the period between population outbreaks the likelihood of significant numbers of common voles being found in secondary habitats is low. Thus, it is considered that the common vole is not necessarily a good choice of focal species to represent small mammals in cereal fields.

Study M-269779-01-1 reports the results of surveys in which small mammal species were monitored within and around winter cereal fields in Germany. The observations confirmed that wood mice, as well as common voles, were the most relevant species and most likely to be captured in cereal fields during spring and summer. Radiotracking confirmed 90th percentile PT values of 1.0 for the wood mouse, thereby demonstrating that the wood mouse is also considered to be a suitable focal species for the refined risk assessment of small herbivorous mammals in cereals. Full details of the study can be found in the study summary.

It is therefore more appropriate to consider the wood mouse as a more representantive focal species to represent small mammals in vineyards for situations in which the common vole is not considered relevant. Using the assumptions of the Tier I risk assessment according to EFSA (2009), the wood mouse scenario passes the risk assessment at Tier I thereby demonstrating acceptable risks to this small mammal species following the proposed uses of Prothioconazole + Spiroxamine EC 460 on cereals.

Combined toxicity

Mixture toxicity and exposure was calculated using the concentration addition model (CA model). For a mixture containing two active substances this can be expressed using the following equation:

$$LD_{50mix\text{-}CA} = 1 / (p^1/LD_{50}^1 + p^2/LD_{50}^2)$$

Where;
$LD_{50mix\text{-}CA}$: calculated mixture toxicity
^{1} and ^{2} indicate active substance 1 and active substance 2, respectively
p: the proportion in the mix of each active as a fraction; Σp should always = 1
LD_{50}: experimentally determined EC_{50}/ LC_{50}/NOEC

For the mixture toxicity risk assessment of Prothioconazole + Spiroxamine EC 460, it is the opinion of the notifier that only an acute risk assessment for combined exposure needs to be presented. Formulations break down into their respective components very shortly after they enter the environment therefore an assessment of the risk to any toxic effects of Prothioconazole + Spiroxamine EC 460 is only applicable to the acute exposure scenario in which the risks to birds and mammals over a very short time period are assessed. The chronic risk assessment considers the potential risks over much longer time periods by which point the formulation will have broken down into the two individual active substances which may then behave differently. Thus, the mixed chronic risk of spiroxamine and prothioconazole applied as Prothioconazole + Spiroxamine EC 460 are considered to be covered by the risk assessments for the individual actives. However, an assessment of the contribution that each active substance makes to the overall chronic mixture toxicity has been presented below.

Careful selection of the toxicity endpoints is necessary for use in the predicted mixture toxicity calculations so that accurate predictions of toxicity can be made as well as meaningful comparisons with the measured formulation toxicity data. For spiroxamine, prothioconazole and prothioconazole-desthio the LD_{50} values of 460, >6200 and 2238 mg a.s./kg bw, respectively have been used in the mixture toxicity calculations. All three of these endpoints have been taken from acute oral toxicity studies with the rat or the mouse and have therefore been conducted using the same test methods. As such, the endpoints are considered suitably comparable for use in a mixture toxicity calculation. It is noted that the endpoint for prothioconazole is an unbound greater than ('>') value and therefore could lead to an inaccurate prediction of the mixture toxicity. However, as the endpoint is a greater than value this would lead to a potential over-estimation of toxicity and therefore would provide a conservative estimate.

Formulation composition

Prothioconazole & Spiroxamine EC 460 has the following composition and relative proportions:

Spiroxamine: 300 g/L (65.2% or 0.652)

Prothioconazole: 160 g/L (34.8% or 0.348)

Predicted mixture toxicity

Using the acute LD_{50} endpoints for spiroxamine, prothioconazole and prothioconazole-desthio of 460, >6200 and 2235 mg a.s./kg bw, respectively, the calculated mix-CA values in terms of total active substance have been determined and presented below. A predicted toxicity value for spiroxamine and prothioconazole has been calculated as well as a predicted value for spiroxamine and prothioconazole-desthio, assuming a worst case 100% conversion of prothioconazole to the more toxic metabolite.

Table CP 10.1.2-20 Calculated acute mammalian oral mixture toxicity in terms of total a.s. content for Prothioconazole & Spiroxamine EC 460

Combination	Endpoint	p^1/LD_{50}	p^2/LD_{50}	Calculated Mix-CA (mg total a.s./kg bw)[3]
Spiroxamine and prothioconazole	LD_{50}	0.00142	0.000561	679
Spiroxamine and prothioconazole-desthio	LD_{50}	0.00142	0.000156	636

[1] proportion of spiroxamine (p = 0.652)
[2] proportion of prothioconazole or prothioconazole-desthio (p = 0.348)
[3] $LD_{50mix\text{-}CA} = 1 / (p^1/LD_{50}^1 + p^2/LD_{50}^2)$
Calculated values have been rounded for presentation purposes.

The predicted mixture toxicity of spiroxamine and prothioconazole in Prothioconazole & Spiroxamine EC 460 is 679 mg total a.s./kg bw. The experimentally determined acute oral LD_{50} was 750 g/kg bw (equivalent to 350 mg total a.s./kg bw). The predicted mixture toxicity value is within a factor of 2 of the experimentally determined value therefore the principles of concentration addition are considered to be appropriate and the predicted value a reliable estimate of toxicity.

The predicted mixture toxicity of spiroxamine and prothioconazole-desthio (assuming complete conversion of prothioconazole to the metabolite) in Prothioconazole & Spiroxamine EC 460 is 636 mg total a.s./kg bw.

Toxic units

The toxic unit (TU) of a mixture is defined as the sum of the TU of each individual substance in the mixture therefore the predicted data can also be examined for the contribution of the two separate active substances to the mixture toxic units.

If the toxicity of the mixture is largely explained by the toxicity of a single active substance, a sufficient protection level might be achieved by simply basing the risk assessment on the toxicity data for that single 'driver'. Hence, where CA provides a reliable estimate of the toxicity of the given mixture and the largest part of the sum of toxic units (*i.e.* ≥ 90 %) calculated for the measured mixture toxicity comes from a single active substance, it can be concluded that this component drives the overall mixture toxicity.

The toxic unit is calculated using the following equation:

$$\sum_{i=1}^{n} TU_i = \sum_{i=1}^{n} \frac{c_i}{ECx_i}$$

Where;
TU_i: Toxic units of component *i*
c_i: concentration of a mixture component *i*
ECx_i: concentration of component *i* provoking x% effect

The calculated toxic units for spiroxamine and prothioconazole along with the percentage contribution of each active to the overall toxicity of the mixture are presented below.

Table CP 10.1.2-21 Toxic unit calculations and contribution of each active substance to the predicted toxicity of Prothioconazole & Spiroxamine EC 460

Organism group	Active substance	C_i/ECx_i	TU_i	ΣTU_i	Contribution (%)
Acute toxicity	Spiroxamine	0.652 / 460	0.00142	0.00147	96.2
	Prothioconazole	0.348 / 6200	0.0000561		3.81
Chronic toxicity	Spiroxamine	0.652 / 21.0	0.0310	0.0347	89.5
	Prothioconazole	0.348 / 95.6	0.00364		10.5

For acute toxicity, spiroxamine is the major contributor to the toxicity of the mixture of spiroxamine and prothioconazole and, as this is greater than the 90% threshold, can be considered to be the driver of the acute toxicity of the mixture. For chronic toxicity it is noted that spiroxamine is also the clear driver of the mixture toxicity with 89.5% (*i.e.* 90%) of the TU_i attributable to spiroxamine. Although a chronic mixture toxicity calculation has not been performed, the chronic risk assessment for spiroxamine on its own would cover the chronic risk assessment for the mixture.

The acute risk assessments for the combined effects of spiroxamine and prothioconazole and that of spiroxamine and prothioconazole-desthio have been presented below. For spiroxamine and prothioconazole the measured toxicity value of 350 mg total a.s./kg bw has been used in place of the predicted toxicity value of 679 mg total a.s./kg bw as it is the most relevant value and also allows for a more conservative assessment.

Mixture toxicity - 1 x 1.25 L/ha application of Prothioconazole & Spiroxamine EC 460

Screening step

Table CP 10.1.2-22 Screening step assessment for acute risk to mammals for the proposed use of Prothioconazole + Spiroxamine 460 in cereals – Combined mixture (SPX & PTZ)

Intended use	Cereals				
Active substance/product	Mixture SPX & PTZ / Prothioconazole + Spiroxamine 460				
Application rate (g total a.s./ha)	1 × 575 (375 SPX + 200 PTZ)				
Acute toxicity (mg total a.s./kg bw)	350				
TER criterion	10				
Crop scenario	Indicator species	SV_{90}	MAF_{90}	DDD_{90} (mg a.s./kg bw/d)	TER_A
Cereals	Small herbivorous mammal	118.4	1.0	68.1	**5.14**

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity exposure ratio; TER values shown in bold fall below the relevant trigger

Table CP 10.1.2-23 Screening step assessment for acute risk to mammals for the proposed use of Prothioconazole + Spiroxamine 460 in cereals – Combined mixture (SPX & PTZ-desthio)

Intended use	Cereals				
Active substance/product	Mixture SPX & PTZ-desthio / Prothioconazole + Spiroxamine 460				
Application rate (g total a.s./ha)	1 × 575 (375 SPX + 200 PTZ)				
Acute toxicity (mg total a.s./kg bw)	636				
TER criterion	10				
Crop scenario	Indicator species	SV_{90}	MAF_{90}	DDD_{90} (mg a.s./kg bw/d)	TER_A
Cereals	Small herbivorous mammal	118.4	1.0	68.1	**9.34**

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity exposure ratio. TER values shown in bold fall below the relevant trigger

Based on the screening step risk assessment for the combined effects of spiroxamine and prothioconazole as well as spiroxamine and prothioconazole-desthio, potential acute risks to mammals have been identified (TER <10) following the proposed use of Prothioconazole + Spiroxamine EC 460 (1 x 1.25 L/ha). A Tier I risk assessment has therefore been presented below.

Tier I Acute risk assessment

Table CP 10.1.2-24 Tier I assessment for acute risk to mammals for the proposed use of Prothioconazole + Spiroxamine 460 in cereals – Combined toxicity (SPX & PTZ)

Intended use	Cereals				
Active substance/product	Mixture SPX & PTZ / Prothioconazole + Spiroxamine 460				
Application rate (g total a.s./ha)	1 × 575 (375 SPX + 200 PTZ)				
Acute toxicity (mg total a.s./kg bw)	350				
TER criterion	10				
Crop scenario Growth stage	Generic focal species	SV_{90}	MAF_{90}	DDD_{90} (mg a.s./kg bw/d)	TER_A
Cereals BBCH >20	Small insectivorous mammal "shrew"	5.4	1.0	3.11	113
Cereals BBCH 30-39	Small omnivorous mammal "mouse"	8.6	1.0	4.95	70.8
Cereals BBCH >40	Small herbivorous mammal "vole"	40.9	1.0	23.5	14.9
Cereals BBCH >40	Small herbivorous mammal "mouse"	5.2	1.0	2.99	117

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio

Table CP 10.1.2-25 Tier I assessment for acute risk to mammals for the proposed use of Prothioconazole + Spiroxamine 460 in cereals – Combined toxicity (SPX & PTZ-desthio)

Intended use	Cereals
Active substance/product	Mixture SPX & PTZ-desthio / Prothioconazole + Spiroxamine 460
Application rate (g total a.s./ha)	1 × 575 (375 SPX + 200 PTZ)
Acute toxicity (mg total a.s./kg bw)	636
TER criterion	10

Crop scenario Growth stage	Generic focal species	SV_{90}	MAF_{90}	DDD_{90} (mg a.s./kg bw/d)	TER_A
Cereals BBCH >20	Small insectivorous mammal “shrew”	5.4	1.0	3.11	205
Cereals BBCH 30-39	Small omnivorous mammal “mouse”	8.6	1.0	4.95	129
Cereals BBCH >40	Small herbivorous mammal “vole”	40.9	1.0	23.5	27.0
Cereals BBCH >40	Small herbivorous mammal “mouse”	5.2	1.0	2.99	213

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio.

Based on the Tier I acute risk assessment for the combined effects of spiroxamine and prothioconazole as well as spiroxamine and prothioconazole-desthio, the acute risks to mammals have been demonstrated to be acceptable (TER ≥10) following the proposed use of Prothioconazole + Spiroxamine EC 460 (1 x 1.25 L/ha). No further acute risk assessment for combined effects is considered to be necessary.

Mixture toxicity - 2 x 1.25 L/ha application of Prothioconazole & Spiroxamine EC 460

Screening step

Table CP 10.1.2-26 Screening step assessment for acute risk to mammals for the proposed use of Prothioconazole + Spiroxamine 460 in cereals – Combined mixture (SPX & PTZ)

Intended use	Cereals
Active substance/product	Mixture SPX & PTZ / Prothioconazole + Spiroxamine 460
Application rate (g total a.s./ha)	2 × 575=375 SPX + 200 PTZ)
Acute toxicity (mg total a.s./kg bw)	350
TER criterion	10

Crop scenario	Indicator species	SV_{90}	MAF_{90}	DDD_{90} (mg a.s./kg bw/d)	TER_A
Cereals	Small herbivorous mammal	118.4	1.2	81.7	**4.28**

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity exposure ratio. TER values shown in bold fall below the relevant trigger

Table CP 10.1.2-27 Screening step assessment for acute risk to mammals for the proposed use of Prothioconazole + Spiroxamine 460 in cereals – Combined mixture (SPX & PTZ-desthio)

Intended use	Cereals				
Active substance/product	Mixture SPX & PTZ-desthio / Prothioconazole + Spiroxamine 460				
Application rate (g total a.s./ha)	2 × 575 (375 SPX + 200 PTZ)				
Acute toxicity (mg total a.s./kg bw)	636				
TER criterion	10				
Crop scenario	Indicator species	SV_{90}	MAF_{90}	DDD_{90} (mg a.s./kg bw/d)	TER_A
Cereals	Small herbivorous mammal	118.4	1.2	81.7	**7.78**

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity exposure ratio. TER values shown in bold fall below the relevant trigger

Based on the screening step risk assessment for the combined effects of spiroxamine and prothioconazole as well as spiroxamine and prothioconazole-desthio, potential acute risks to mammals have been identified (TER <10) following the proposed use of Prothioconazole + Spiroxamine EC 460 (2 x 1.25 L/ha). A Tier I risk assessment has therefore been presented below.

Tier I Acute risk assessment

Table CP 10.1.2-28 Tier I assessment for acute risk to mammals for the proposed use of Prothioconazole + Spiroxamine 460 in cereals – Combined toxicity (SPX & PTZ)

Intended use	Cereals				
Active substance/product	Mixture SPX & PTZ / Prothioconazole + Spiroxamine 460				
Application rate (g total a.s./ha)	2 × 575 (375 SPX + 200 PTZ)				
Acute toxicity (mg total a.s./kg bw)	350				
TER criterion	10				
Crop scenario Growth stage	Generic focal species	SV_{90}	MAF_{90}	DDD_{90} (mg a.s./kg bw/d)	TER_A
Cereals BBCH >20	Small insectivorous mammal "shrew"	5.4	1.2	3.73	93.9
Cereals BBCH 30-39	Small omnivorous mammal "mouse"	8.6	1.2	5.93	59.0
Cereals BBCH >40	Small herbivorous mammal "vole"	40.9	1.2	28.2	12.4
Cereals BBCH >40	Small herbivorous mammal "mouse"	5.2	1.2	3.59	97.6

SV: shortcut value; MAF: multiple application factor; DDD: daily dietary dose; TER: toxicity to exposure ratio

Table CP 10.1.2-29 Tier I assessment for acute risk to mammals for the proposed use of Prothioconazole + Spiroxamine 460 in cereals – Combined toxicity (SPX & PTZ-desthio)

Intended use	Cereals
Active substance/product	Mixture SPX & PTZ-desthio / Prothioconazole + Spiroxamine 460
Application rate (g total a.s./ha)	2 × 575 (375 SPX + 200 PTZ)
Acute toxicity (mg total a.s./kg bw)	636
TER criterion	10

Crop scenario Growth stage	Generic focal species	SV_{90}	MAF	DDD_{90} (mg a.s./kg bw/d)	TER_A
Cereals BBCH >20	Small insectivorous mammal "shrew"	5.4	1.2	3.73	171
Cereals BBCH 30-39	Small omnivorous mammal "mouse"	8.6	1.2	5.93	107
Cereals BBCH >40	Small herbivorous mammal "vole"	40.9	1.2	28.2	22.5
Cereals BBCH >40	Small herbivorous mammal "mouse"	5.2	1.2	3.59	177

SV: shortcut value; MAF: multiple application factor; DDD: daily dietary dose; TER: toxicity to exposure ratio

Based on the Tier I acute risk assessment for the combined effects of spiroxamine and prothioconazole as well as spiroxamine and prothioconazole-desthio, the acute risks to mammals have been demonstrated to be acceptable (TER ≥ 10) following the proposed use of Prothioconazole + Spiroxamine EC 460 (2 x 1.25 L/ha). No further acute risk assessment for combined effects is considered to be necessary.

Risks for mammals through drinking water

In addition to dietary items, mammals may also be exposed to residues occurring in drinking water. The daily dietary dose (DDD) values used in the standard dietary risk assessments do not encompass drinking water and therefore the potential risk from this exposure route has not been covered in the dietary risk assessment.

The puddle scenario is relevant for mammals and considers puddles occurring on the soil surface following a rainfall event after application and is considered possible in all crop types.

In accordance with the EFSA Guidance Document (2009), based on the characteristics of the exposure scenario and standard assumptions for water uptake by animals no specific requirement for drinking water exposure calculation and TER determination based on the puddle scenario is required:

- for substances with a Koc <500 L/kg (less sorptive); if the ratio of application rate (g a.s./ha) to effective endpoint mg a.s./kg bw/d does not exceed 50;
- for substances with a Koc ≥ 500 L/kg (more sorptive); if the ratio of application rate (g a.s./ha) to effective endpoint mg a.s./kg bw/d does not exceed 3000.

The geomean Koc for spiroxamine is 4111 L/kg. For prothioconazole and prothioconazole-desthio, mean Koc values of 1765 L/kg and 575 L/kg, respectively have been used (EFSA Scientific Report (2007) 106, 1-98). Thus, spiroxamine, prothioconazole and prothioconazole-desthio all belong to the group of more sorptive substances.

For spiroxamine the ratio calculations are based on two applications of 375 g a.s./ha. For prothioconazole and prothioconazole-desthio the ratio calculations are based on two applications of 200 g a.s./ha.

Table CP 10.1.2-30 Ratios of effective application rate (AR_{eff}) to acute and long-term endpoints for spiroxamine, prothioconazole and prothioconazole-desthio following the proposed use of Prothioconazole & Spiroxamine EC 460 - puddle scenario

Test substance	AR_{eff} (g/ha)[a]	Toxicological endpoint (mg a.s./kg bw/d)	Ratio (AR_{eff}/endpoint)	Trigger
Acute				
Spiroxamine	450	LD_{50} 460	0.978	3000
Prothioconazole	240	LD_{50} >6200	0.0387	
Prothioconazole-desthio	240	LD_{50} 2235	0.107	
Long-term				
Spiroxamine	525	NOAEL 21.0	25.0	3000
Prothioconazole	280	NOEL 95.6	2.93	
Prothioconazole-desthio	280	NOEL 10	28.0	

[a] AR_{eff} = based on an application rate of 375 or 200 g a.s./ha for spiroxamine or prothioconazole / prothioconazole-desthio, respectively with a MAF of 1.2 and 1.4 applied for acute and reproductive risk assessments, respectively

The ratios for both acute and reproductive risks are below the relevant trigger of 3000 for spiroxamine, prothioconazole and prothioconazole-desthio therefore a quantitative risk assessment is not necessary. Thus, there are no unacceptable risks to mammals from exposure to either spiroxamine, prothioconazole or prothioconazole-desthio *via* drinking water.

Secondary poisoning

The Log P_{ow} of spiroxamine is 2.79 and 2.98 at pH 7 for diastomers A and B, respectively but at pH 9 these value are 4.88 and 5.08, respectively. Thus the trigger value of 3 for a secondary poisoning risk assessment is met.

The Log Pow of prothioconazole was determined to be 3.82 at pH 7. Thus a risk assessment for a generic earthworm eating mammal and a generic fish eating mammal has been performed to evaluate the risk of secondary poisoning.

<u>Consideration of secondary poisoning risk due to metabolites</u>

The Log P_{ow} of spiroxamine-desethyl (M01) is 2.41, 1.97 and >3.64 at pH 4, 7 and 9, respectively. The Log P_{ow} of spiroxamine-despropyl (M02) is 1.95, 1.41 and >3.44 at pH 4, 7 and 9, respectively. The Log P_{ow} of spiroxamine-N-oxide (M03) is 2.82, 2.59 and 2.63 at pH 4, 7 and 9, respectively. The Log P_{ow} of spiroxamine-carboxylic acid (M06) is 0.45, -0.25 and 0.10 at pH 4, 7 and 9, respectively. Thus, an assessment of the potential risk from bioconcentration of spiroxamine-desethyl (M01) and spiroxamine-despropyl (M02) also needs to be addressed in the risk assessment.

Prothioconazole-desthio and prothioconazole-S-methyl are the major soil and major aquatic metabolites of prothioconazole. Both metabolites have log Pow values >3, *i.e.* 3.04 and 4.30, respectively. Therefore the risk of secondary poisoning for earthworm eating and for fish eating mammals has been considered for both metabolites. Another major aquatic metabolite of prothioconazole, 1,2,4-triazole, has a log Pow of <3, therefore no risk to mammals due to bioaccumulation has to be expected from this metabolite.

<u>Risk assessment for earthworm-eating mammals *via* secondary poisoning</u>

According to EFSA (2009), the risk for vermivorous mammals is assessed for a small mammal of 10 g body weight with a daily food consumption of 12.8 g. Bioaccumulation in earthworms is estimated based on predicted concentrations in soil.

To achieve a concise risk assessment, the risk envelope approach is applied whereby the maximum application rate of 2 x 1.25 L/ha has been considered. For spiroxamine, M01 and M02, the $PEC_{soil\ accumulation}$ or the 21-day TWA PEC_{soil} value, whichever is highest, has been used in the risk assessment. There are no mammalian reproductive toxicity data available for M01 and M02, therefore the NOAEL of 21.0 mg/kg bw/day for spiroxamine has been used as a surrogate value.

For prothioconazole, prothioconazole-desthio and prothioconazole-S-methyl, the 21-day TWA PEC_{soil} values have been taken from the spiroxamine RAR (Spiroxamine dRAR, Volume 3, Annex B.9) along with the Log P_{ow} values. K_{oc} values and the toxicity endpoints have been taken from the EFSA Conclusion for Prothioconazole (EFSA Scientific Report (2007) 106, 1 – 98). For Prothioconazole-S-methyl there are no mammalian reproductive toxicity data therefore the prothioconazole endpoint has been used as a surrogate.

The secondary poisoning risk assessments for earthworm-eating mammals from exposure to spiroxamine, KWG 4168-desethyl (M01), KWG 4168-despropyl (M02), prothioconazole, prothioconazole-desthio and prothioconazole-S-methyl are presented in the tables below.

Table CP 10.1.2-31 Assessment of the risk for earthworm-eating mammals due to exposure to spiroxamine *via* bioaccumulation in earthworms (secondary poisoning)

Parameter	Spiroxamine	Comments
PEC_{soil} (mg a.s./kg soil)	0.157	21-day TWA PEC_{soil}
Log P_{ow} / P_{ow}	4.0 / 10000	Mean value of 4.0 has been used based on the values for diastomers A and B at pH 7 and 9
K_{oc}	4111	Mean
f_{oc}	0.02	Default
BCF_{worm}	1.47	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw}) = (0.84 + 0.012 \times P_{ow}) / f_{oc} \times K_{oc}$
PEC_{worm}	0.231	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	0.295	$DDD = PEC_{worm} \times 1.28$
NOEL (mg/kg bw/d)	21.0	M-762441-01-1
TER_{LT}	71.1	Acceptable risks (TER>5)

Table CP 10.1.2-32 Assessment of the risk for earthworm-eating mammals due to exposure to spiroxamine-desethyl (M01) *via* bioaccumulation in earthworms (secondary poisoning)

Parameter	Spiroxamine-desethyl	Comments
PEC_{soil} (mg/kg soil)	0.030	Accumulation PEC_{soil} used as worst-case
Log P_{ow} / P_{ow}	3.64 / 4365	-
K_{oc}	3271	Mean
f_{oc}	0.02	Default

Parameter	Spiroxamine-desethyl	Comments
BCF_{worm}	0.814	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw}) = (0.84 + 0.012 \times P_{ow}) / f_{oc} \times K_{oc}$
PEC_{worm}	0.0244	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	0.0312	$DDD = PEC_{worm} \times 1.28$
NOEL (mg/kg bw/d)	21.0	Value determined for spiroxamine used as a surrogate
TER_{LT}	672	Acceptable risks (TER>5)

Table CP 10.1.2-33 Assessment of the risk for earthworm-eating mammals due to exposure to spiroxamine-despropyl (M02) *via* bioaccumulation in earthworms (secondary poisoning)

Parameter	Spiroxamine-despropyl	Comments
PEC_{soil} (mg/kg soil)	0.021	Accumulation PEC_{soil} used as worst-case
$Log P_{ow}$ / P_{ow}	3.44 / 2754	-
K_{oc}	2695	Mean
f_{oc}	0.02	Default
BCF_{worm}	0.629	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw}) = (0.84 + 0.012 \times P_{ow}) / f_{oc} \times K_{oc}$
PEC_{worm}	0.0132	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	0.0169	$DDD = PEC_{worm} \times 1.28$
NOEL (mg/kg bw/d)	21.0	Value determined for spiroxamine used as a surrogate
TER_{LT}	1243	Acceptable risks (TER>5)

Table CP 10.1.2-34 Assessment of the risk for earthworm-eating mammals due to exposure to prothioconazole *via* bioaccumulation in earthworms (secondary poisoning)

Parameter	Prothioconazole	Comments
PEC_{soil} (mg a.s./kg soil)	0.028	21-day TWA PEC_{soil} from spiroxamine RAR
$Log P_{ow}$ / P_{ow}	3.82 / 6607	Spiroxamine RAR
K_{oc}	1765	Mean value taken from EFSA Scientific Report (2007) 106, 1-98
f_{oc}	0.02	Default
BCF_{worm}	2.27	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw}) = (0.84 + 0.012 \times P_{ow}) / f_{oc} \times K_{oc}$
PEC_{worm}	0.0636	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$

Daily dietary dose (mg a.s./kg bw/d)	0.0813	DDD = PEC_{worm} × 1.28
NOEL (mg a.s./kg bw/d)	95.6	EFSA Scientific Report (2007) 106, 1-98
TER_{LT}	1175	Acceptable risks (TER>5)

Table CP 10.1.2-35 Assessment of the risk for earthworm-eating mammals due to exposure to Prothioconazole-desthio *via* bioaccumulation in earthworms (secondary poisoning)

Parameter	Prothioconazole-desthio	Comments
PEC_{soil} (mg/kg soil)	0.066	21-day TWA PEC_{soil} from spiroxamine RAR
Log P_{ow} / P_{ow}	3.04 / 1096	Spiroxamine RAR
K_{oc}	575	Mean value taken from EFSA Scientific Report (2007) 106, 1-98
f_{oc}	0.02	Default
BCF_{worm}	1.22	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw})$ $= (0.84 + 0.012 \times P_{ow}) / f_{oc} \times K_{oc}$
PEC_{worm}	0.0803	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	0.103	DDD = PEC_{worm} × 1.28
NOEL (mg/kg bw/d)	10	EFSA Scientific Report (2007) 106, 1-98
TER_{LT}	97.2	Acceptable risks (TER>5)

Table CP 10.1.2-36 Assessment of the risk for earthworm-eating mammals due to exposure to Prothioconazole-S-methyl *via* bioaccumulation in earthworms (secondary poisoning)

Parameter	Prothioconazole-S-methyl	Comments
PEC_{soil} (mg/kg soil)	0.018	21-day TWA PEC_{soil} from spiroxamine RAR
Log P_{ow} / P_{ow}	4.30 / 19953	Spiroxamine RAR
K_{oc}	2556	Mean value taken from EFSA Scientific Report (2007) 106, 1-98
f_{oc}	0.02	Default
BCF_{worm}	4.70	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw})$ $= (0.84 + 0.012 \times P_{ow}) / f_{oc} \times K_{oc}$
PEC_{worm}	0.0846	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	0.108	DDD = PEC_{worm} × 1.28
NOEL (mg/kg bw/d)	95.6	Value determined for prothioconazole used as a surrogate

TER_{LT}	883	Acceptable risks (TER>5)

For the secondary poisoning risk assessments for earthworm-eating mammals from exposure to spiroxamine, M01, M02, prothioconazole, prothioconazole-desthio and prothioconazole-S-methyl the TER values are >5 thereby demonstrating an acceptable risk to mammals *via* this route of exposure.

Risk assessment for fish-eating mammals *via* secondary poisoning

According to EFSA (2009), the risk for piscivorous mammals is assessed for a mammal of 3000 g body weight with a daily food consumption of 425 g. Bioaccumulation in fish is estimated based on predicted concentrations in surface water.

Once again, to achieve a concise risk assessment, the risk envelope approach is applied. The highest Step 3 TWA PEC_{sw} of 2.029 µg a.s./L for spiroxamine has been used in the risk assessment. For M01 the highest Step 2 PEC_{sw} value of 0.826 µg/L has been used and for M02 the highest Step 2 PEC_{sw} value of 0.699 µg/L has been used. For M01 and M02 there are no BCF values available therefore the BCF value determined for spiroxamine (87 L/kg) has been used. Furthermore, there are no mammalian reproductive toxicity data available for M01 and M02, therefore the NOAEL of 21.0 mg/kg bw/day for spiroxamine has been used as a surrogate value.

For prothioconazole the highest Step 2 PEC_{sw} value of 1.263 µg a.s./L has been used. For prothioconazole-desthio the highest Step 3 max PEC_{sw} value of 1.244 µg/L has been used and for prothioconazole-S-methyl the highest Step 2 TWA PEC_{sw} value of 0.542 µg/L has been used. These values have been taken from the current draft RAR for Spiroxamine (Spiroxamine dRAR, Volume 3, Annex B.9). The fish BCF values for prothioconazole and prothioconazole-desthio of 19.7 and 65 L/kg, respectively have been used with NOEL values of 95.6 and 10 mg/kg bw/day, respectively. For prothioconazole-S-methyl there are no BCF or mammalian reproductive toxicity data available therefore the values for prothioconazole have been used as a surrogate.

The secondary poisoning risk assessments for fish-eating mammals from exposure to spiroxamine, KWG 4168-desethyl (M01), KWG 4168-despropyl (M02), prothioconazole, prothioconazole-desthio and prothioconazole-S-methyl are presented in the tables below.

Table CP 10.1.2-37 Assessment of the risk for fish-eating mammals due to exposure to spiroxamine *via* bioaccumulation in fish (secondary poisoning)

Parameter	Spiroxamine	Comments
PEC_{sw} (mg a.s./L)	0.002029	FOCUS Step 3 TWA PEC_{sw} (calculated for Spring cereals: D1 ditch, 2 x 375 g a.s./ha, early application)
PEC_{water} (mg a.s./L)	0.002029	TWA PEC_{sw} value used
BCF_{fish}	87	From study M-006479-01-1
PEC_{fish}	0.177	$PEC_{fish} = PEC_{water} \times BCF_{fish}$
Daily dietary dose (mg a.s./kg bw/d)	0.0251	$DDD = PEC_{fish} \times 0.142$
NOAEL (mg a.s./kg bw/d)	21.0	M-762441-01-1
TER_{LT}	838	Acceptable risks (TER>5)

Table CP 10.1.2-38 Assessment of the risk for fish-eating mammals due to exposure to spiroxamine-desethyl (M01) *via* bioaccumulation in fish (secondary poisoning)

Parameter	Spiroxamine-desethyl	Comments
PEC_{sw} (max) (mg/L)	0.000826	FOCUS Step 2 maximum PEC_{sw} (calculated for Spring/Winter cereals; 2 x 375 g a.s./ha)
PEC_{water}	0.000438	$PEC_{water} = max PEC_{sw} \times f_{twa}$, where $f_{twa} = 0.53$; in line with approach in EFSA (2009)
BCF_{fish}	87	Value determined for spiroxamine used as a surrogate
PEC_{fish}	0.0381	$PEC_{fish} = PEC_{water} \times BCF_{fish}$
Daily dietary dose (mg/kg bw/d)	0.00541	$DDD = PEC_{fish} \times 0.142$
NOAEL (mg/kg bw/d)	21.0	Value determined for spiroxamine used as a surrogate
TER_{LT}	3883	Acceptable risks (TER>5)

Table CP 10.1.2-39 Assessment of the risk for fish-eating mammals due to exposure to spiroxamine-despropyl (M02) *via* bioaccumulation in fish (secondary poisoning)

Parameter	Spiroxamine-despropyl	Comments
PEC_{sw} (max) (mg/L)	0.000699	FOCUS Step 2 maximum PEC_{sw} (calculated for Spring/Winter cereals; 2 x 375 g a.s./ha)
PEC_{water}	0.000370	$PEC_{water} = max PEC_{sw} \times f_{twa}$, where $f_{twa} = 0.53$; in line with approach in EFSA (2009)
BCF_{fish}	87	Value determined for spiroxamine used as a surrogate
PEC_{fish}	0.0322	$PEC_{fish} = PEC_{water} \times BCF_{fish}$
Daily dietary dose (mg/kg bw/d)	0.00458	$DDD = PEC_{fish} \times 0.142$
NOAEL (mg/kg bw/d)	21.0	Value determined for spiroxamine used as a surrogate
TER_{LT}	4588	Acceptable risks (TER>5)

Table CP 10.1.2-40 Assessment of the risk for fish-eating mammals due to exposure to prothioconazole *via* bioaccumulation in fish (secondary poisoning)

Parameter	Prothioconazole	Comments
PEC_{sw} (max) (mg/L)	0.001263	FOCUS Step 2 TWA PEC_{sw} (calculated for cereals)
PEC_{water}	0.001263	TWA PEC_{sw} value used

BCF_{fish}	19.7	EFSA Scientific Report (2007) 106, 1-98
PEC_{fish}	0.0249	$PEC_{fish} = PEC_{water} \times BCF_{fish}$
Daily dietary dose (mg/kg bw/d)	0.00353	$DDD = PEC_{fish} \times 0.142$
NOEL (mg/kg bw/d)	95.6	EFSA Scientific Report (2007) 106, 1-98
TER_{LT}	27058	Acceptable risks (TER>5)

Table CP 10.1.2-41 Assessment of the risk for fish-eating mammals due to exposure to Prothioconazole-desthio *via* bioaccumulation in fish (secondary poisoning)

Parameter	Prothioconazole-desthio	Comments
PEC_{sw} (max) (mg/L)	0.001244	FOCUS Step 3 maximum PEC_{sw} (Winter cereals, R4 Stream)
PEC_{water}	0.000659	$PEC_{water} = \max PEC_{sw} \times f_{twa}$, where $f_{twa} = 0.53$; in line with approach in EFSA (2009)
BCF_{fish}	65	EFSA Scientific Report (2007) 106, 1-98
PEC_{fish}	0.0429	$PEC_{fish} = PEC_{water} \times BCF_{fish}$
Daily dietary dose (mg/kg bw/d)	0.00609	$DDD = PEC_{fish} \times 0.142$
NOEL (mg/kg bw/d)	10	EFSA Scientific Report (2007) 106, 1-98
TER_{LT}	1643	Acceptable risks (TER>5)

Table CP 10.1.2-42 Assessment of the risk for fish-eating mammals due to exposure to Prothioconazole-S-methyl *via* bioaccumulation in fish (secondary poisoning)

Parameter	Prothioconazole-S-methyl	Comments
PEC_{sw} (max) (mg/L)	0.000542	FOCUS Step 2 TWA PEC_{sw} (calculated for cereals)
PEC_{water}	0.000542	TWA PEC_{sw} value used
BCF_{fish}	19.7	Value determined for prothioconazole used as a surrogate
PEC_{fish}	0.0107	$PEC_{fish} = PEC_{water} \times BCF_{fish}$
Daily dietary dose (mg/kg bw/d)	0.00152	$DDD = PEC_{fish} \times 0.142$
NOEL (mg/kg bw/d)	95.6	Value determined for prothioconazole used as a surrogate
TER_{LT}	63053	Acceptable risks (TER>5)

For the secondary poisoning risk assessments for fish-eating mammals from exposure to spiroxamine, M01, M02, prothioconazole, prothioconazole-desthio and prothioconazole-S-methyl the TER values are all >5 thereby demonstrating an acceptable risk to mammals *via* this route of exposure.

Biodiversity

No relevant scientifically peer-reviewed open literature could be found on spiroxamine or its major metabolites, from an ecotoxicological perspective, on mammals. Therefore, it is considered that the potential impact of the active substance on biodiversity and the ecosystem, including potential indirect effects *via* alteration of the food web, are covered by the risk assessment for mammals in this section and in the ED hazard assessment.

CP 10.1.2.1 Acute oral toxicity to mammals

Please refer to Document M-CP Section 7 Toxicology for a summary of the acute oral rat study using Prothioconazole + Spiroxamine EC 460 (M-078810-02-1).

CP 10.1.2.2 Higher tier data on mammals

The following summary relates to a report which has been submitted in order to justify the selection of the ecotoxicologically relevant NOAEL used in the chronic mammalian risk assessment.

Data Point:	KCP 10.1.2.2/02
Report Author:	[redacted]
Report Year:	2021
Report Title:	Spiroxamine: Consideration of long-term mammalian toxicology endpoints for the bird and mammal risk assessment
Report No:	0471838-TOX3
Document No:	M-762441-01-1
Guideline(s) followed in study:	None
Deviations from current test guideline:	None
Previous evaluation:	No, not previously submitted -
GLP/Officially recognised testing facilities:	not applicable
Acceptability/Reliability:	Yes

Executive Summary

The available long-term Toxicology data has been reviewed in order to determine an ecotoxicologically relevant endpoint for the mammalian risk assessment. A NOAEL of 21.0 mg a.s./kg bw/day has been selected and considered to be appropriate.

I. Methods

The EFSA 2009 Bird & Mammal Guidance Document[1] sets out a different approach to determining ecotoxicologically relevant toxicity end points for wild birds and mammal risk assessments. The philosophy of these guidelines is to set a toxicity endpoint which would protect the dynamics of the population, rather than protecting any individual against an effect, perhaps inconsequential, to their health, survival or reproduction. According to the outcome of the pesticides peer review meeting on recurring issues in ecotoxicology (EFSA PPR meeting 133 September 2015[2]), an ecotoxicologically

[1] European Food Safety Authority; Guidance Document on Risk Assessment for Birds & Mammals on request from EFSA. EFSA Journal 2009; 7(12):1438. doi:10.2903/j.efsa.2009.1438

[2] EFSA (European Food Safety Authority), 2015. Technical report on the outcome of the pesticides peer review meeting on general recurring issues in ecotoxicology. EFSA supporting publication 2015:EN-924. 62 pp

relevant endpoint should be set in collaboration with mammalian toxicologists and should be used in all the steps of the risk assessment. The available long-term data were assessed and considered in the table below.

II. Results and Discussion

The table below presents the results achieved in the long-term Toxicology studies.

Table CP 10.1.2.2/02-1 Consideration of Toxicology data for derivation of an ecotoxicologically relevant endpoint for risk assessment

Endpoint
Body weight change, behavioural effects and systemic toxicity : • NOAEL 125 ppm (equivalent ♂/♀: 1.9/2.7 mg/kg bw/day) based on systemic toxicity (hyperkeratosis of oesophagus and forestomach) and clinical chemistry paratmeters (↑ total cholesterol) [90 day oral (dietary) toxicity study in the rat], with the LOAEL of 625 ppm (♂/♀: 9.3/13.2 mg/kg bw/day). • NOAEL 80 ppm (equivalent to ♂/♀: 5.5/6.7 mg/kg bw/day) based on ↓ parental body weight gain in the 2008 two-generation study with evidence of systemic toxicity (hyperkeratosis of oesophagus).
Indices of gestation, litter size, pup and litter weight • NOAEL 80 ppm (F_1/F_2: 6.5/6.7 mg/kg bw/day) based on ↓ pup body weights in the 2008 two-generation study.
Indices of viability, pre- and post-implantation loss: • NOAEL of 300 ppm (♂/♀: 21.0/21.2 mg/kg bw/day), the highest dose tested in the 2008 two-generation study, with no effects on viability index or reproduction endpoints • In the rat developmental dose range finder toxicity study (implantations, *corpora lutea* were all decreased, pre- and post implantation loss were increased with litter viability decreased with LOAEL of 150 mg/kg bw/day, with an NOAEL of 100 mg/kg bw/day. • In a further rat developmental toxicity study confirmed no effect on implantations, *corpora lutea*, pre- or post implantation loss or litter viability, with a NOAEL 100 mg/kg bw/day. • Pre- and post implantation loss was increased in the rabbit developmental dose range-finder study NOAEL of 75 mg/kg bw/day (LOAEL 100 mg/kg bw/day), without any effect on litter viability. These effects occured in the presence of maternal toxicity (reduced body weight, body weight, food consumption, gastric ulceration, reduced faecal output). • In the rabbit developmental main toxicity study no effect on implantations, *corpora lutea*, pre- or post implantation loss or litter viability were observed , with a NOAEL 80 mg/kg bw/day.
Embryo/fetal toxicity including teratological effects: • NOAEL (Embryo/fetal toxicity) of 80 mg/kg bw/day based on no embryo/fetal toxicity in the rabbit main study with a NOAEL 80 mg/kg bw/day. In the rat an NOAEL of 100 mg/kg bw/day was obtained in the presence of reduced fetal body weight. • NOAEL (teratological effects) of 80 mg/kg bw/day based increased incidence of palatoschisis (cleft palate) in the rat in the presence of maternal toxicity (↓ body weight, and body weight, food consumption, clinical signs). • In the rabbit, a NOAEL of 20 mg/kg bw/day was observed based on increased incidence of spontaneous malformations (conjoined sternebrae, caudal displacement of ears) in the presence of maternal toxicity. These malformations, observed at 80 mg/kg bw/day (a dose level deemed to exceed the maximum tolerated dose) were deemed both incidental and occurred in the presence of overt maternal toxicity, rather than evidence of direct teratogenic effect of the compound. Unlike the rat, palatoschisis (cleft palate) was not observed.
Number aborting and number delivering early: • No evidence of abortions up to doses of 100 mg/kg bw/day in the rat (where examined at the highest dose level) or 80 mg/kg bw/day in the rabbit.
Systemic toxicity and effects on adult body weight:

Endpoint
• NOAEL 125 ppm (equivalent ♂/♀: 1.9/2.7 mg/kg bw/day) based on systemic toxicity (hyperkeratosis of oesophagus and forestomach) and clinical chemistry paratmeters (↓ total cholesterol) [90 day oral (dietary) toxicity study in the rat (1)], with the LOAEL of 625 ppm (♂/♀: 9.3/13.2 mg/kg bw/day). • NOAEL 80 ppm (equivalent to ♂/♀: 5.5/6.7 mg/kg bw/day) based on ↓ parental body weight gain in the 2008 two-generation study with evidence of systemic toxicity (hyperkeratosis of oesophagus).
Indices of post-natal growth, indices of lactation and data on physical landmarks: • Evidence of effects on developmental landmarks were observed in the 2008 two-generation study. Both prepuptial separation and vaginal opening were delayed in F_1 offspring, with no effect on anogenital distance in F_2. Developmental landmarks were independent of offspring weight reductions, not endocrine driven but occured in the presence or maternal toxicity (↓ body weight gain, hyperkeratosis of the oesophagus observed at necropsy due to irritation of the digestive tract causing chronic maternal stress). A NOAEL of 80 ppm (F_1/F_2: 6.5/6.7 mg/kg bw/day) based on ↓ pup bodyweights in the 2008 two-generation study.
Survival and general toxicity up to sexual maturity: • NOAEL of 80 ppm (F_1/F_2: 6.5/6.7 mg/kg bw/day) based on ↓ pup bodyweights in the 2008 two-generation study, with no on survival up to the top dose of 300 ppm (F_1/F_2: 22.2/27.7 mg/kg bw/day) 2008 two-generation study. • NOAEL 125 ppm (equivalent ♂/♀: 1.9/2.7 mg/kg bw/day) based on systemic toxicity (hyperkeratosis of oesophagus and forestomach) and clinical chemistry paratmeters (↓ total cholesterol) [90 day oral (dietary) toxicity study in the rat], with the LOAEL of 625 ppm (♂/♀: 9.3/13.2 mg/kg bw/day).

With regard to the selection of endpoints for the long-term assessment, it is considered that effects on populations will not occur if the survival rate, reproduction rate and development of individuals are not affected. Therefore, in principle, only endpoints in toxicity tests which are related to these key factors of population dynamics are ecotoxicologically relevant.

The refinement for the two-generation study (M-304231-01-1) is based on the assumption that the effects reported at highest dose level, 300 ppm do not influence the population success and the total reproductive outcome of mammals in treated areas. At this dose parental animals showed slight decreases of body weight (up to ↓8.3%) or body weight gain (up to ↓14.2%) as well as irritation induced hyperkeratosis of the oesophagus epithelium. However there were delays to developmental milestones of reaching puberty, i.e. preputial separation (PPS) in males and vaginal opening (VO) in females, in the F1 offspring only which were apparently treatment related. These effects occurred only in the presence of maternal toxicity (reduced body weight gain and hyperkeratosis of the oesophagus observed at necropsy due to irritation of the digestive tract causing chronic maternal stress).

The mean time of attainment of PPS was 42.0, 42.3, 42.8 and 44.6 days in 0, 20, 80 and 300 ppm groups, respectively, the delay at 300 ppm being statistically significant ($p<0.01$). The mean time of attainment of VO was 34.3, 34.6, 35.2 and 38.4 days in 0, 20, 80 and 300 ppm groups, respectively, the delay at 300 ppm being statistically significant ($p<0.01$). To understand if these delays in attainment of VO and PPS at 300 ppm were attributed to the marginal decreases in body weight effects, an analysis of covariance for the day of attainment of PPS and VO versus male and female pup body weight on post-natal day (PND 21), respectively, was undertaken.

Analysis of covariance for the day of attainment of PPS versus male pup body weight on PND 21 confirmed that the delay in PPS could not account for differences in PND 21 pup body weight. A similar conclusion was also reached for the day of attainment of VO versus female pup body weight on PND 21. Therefore, it cannot be concluded that differences in PND 21 pup body weight accounts for differences in PPS or VO.

III. Conclusion

In conclusion, the most plausible explanation of the effects observed in the two-generation study is that they are all directly caused by, or are secondary to, the systemic toxicity of spiroxamine in parental

females. As the effects are relatively small compared to the control, these are considered to have no effect at the population level and are therefore not considered to be ecologically relevant.

The resultant long-term effects and chronic endpoint is addressed with the NOAEL for reproductive toxicity assessment of 21.0 mg/kg bw/day.

Assessment and conclusion by applicant:

This report presents an assessment of the available mammalian toxicology data with spiroxamine along with a summary of the specific effects seen for various parameters for each study. A NOAEL of 21.0 mg a.s./kg bw/day has been determined from the rat two generation study and considered suitable for ecotoxicological risk assessment of wild mammals. The refinement is based on the assumption that the effects reported at the 300 ppm top dose level do not influence the population success and the total reproductive outcome of mammals in treated areas. At this dose parental animals showed slight decreases of body weight (up to 8.3 %) or body weight gain (up to 14.2 %) as well as irritation induced hyperkeratosis of the oesophagus epithelium. There were delays to developmental milestones of reaching puberty, *i.e.* preputial separation (PPS) in males and vaginal opening (VO) in females, in the F_1 offspring only which were apparently treatment related, but these were relatively small and are not considered to have an adverse effect at the population level. It is noted that in the same study the reproductive parameters (mating, fertility, oestrous cycling, sperm motility, sperm count, sperm morphology, pregnancy, natural delivery, litter observations, mean ovarian follicles, *corpora lutea*) were unaffected at the highest dose therefore it has been demonstrated that these small delays in PPS and VO do not have an adverse effect on the parameters that are considered to be relevant at the population level.

Thus, the lowest ecotoxicologically relevant NOAEL, suitable for use in the mammalian reproductive risk assessment, was considered to be 21.0 mg a.s./kg bw/day.

The report is considered to be acceptable.

Ecological data

The following data are considered relevant to the proposed use of Prothioconazole + Spiroxamine EC 460 in cereals.

Data Point:	KCP 10.1.2.2/03
Report Author:	[redacted]
Report Year:	2006
Report Title:	Generic field monitoring of mammals on cereal fields in spring and summer in Germany
Report No:	BARTS 030_
Document No:	M-369779-01-1
Guideline(s) followed in study:	The test was specifically designed for this study
Deviations from current test guideline:	None
Previous evaluation:	Yes, evaluated and accepted RAR (2010)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

In this generic study, small mammal species (rodents) were monitored on four study plots within and around winter cereal fields. On each plot a grid of 64 life traps was installed where traps were set up in

the cereal field as well as in the adjacent surrounding. The abundance of small mammals was investigated by live trapping (capture – mark - recapture method). Furthermore, individual wood mice and common voles were radio-tracked continuously from dusk till dawn (wood mice) and over 24 hours (common voles). From the telemetry data the portion of time/potential foraging time in cereal fields, the habitat preference (Jacob's index) and individual home ranges were calculated.

The observations confirmed that wood mice and common voles were the most relevant species and most likely to be captured in cereal fields during spring and summer.

Study area

This generic field study has been conducted in and around winter cereal fields in the region of [redacted]. This region is a typical area for cereal cultivation in Europe.

I. Methods

The study was carried out 21 March and 31 August 2005.

Small mammal species (rodents) were monitored on four study plots within and around winter cereal fields. On each plot a grid of 64 life traps was installed where traps were set up in the cereal field as well as in the adjacent surrounding. The abundance of small mammals was investigated by live trapping (capture – mark - recapture method). Furthermore, individual wood mice and common voles were radio-tracked continuously from dusk till dawn (wood mice) and over 24 hours (common voles). From the telemetry data the portion of time/potential foraging time in cereal fields, the habitat preference (Jacob's index) and individual home ranges were calculated.

For evaluation and reporting the observations were related to the following three BBCH groups: BBCH group 1 comprised early post-emergence and tillering stages (BBCH principal growth stages 1-2), BBCH group 2 included stem elongation and flowering (BBCH principal growth stages 3-6), and BBCH group 3 combined fruiting and ripening stages until just before harvest (BBCH principal growth stages 7-9).

II. Results and Discussion

Table CP 10.1.2.2/01-1 Overview of relevant species, habitat and PT

Relevant species in the cereal field habitat (based on live-trapping)				
Species	**Mean trapping rate (captures/100 trappings)**		**Captures in the cereal field [%]**	
	Cereal field (based on 15168 trapnights)	**Surrounding (based on 5056 trapnights)**		
Wood mouse (*Apodemus sylvaticus*)	2.51	1.17	68.21	
Common vole (*Microtus arvalis*)	1.61	0.89	83.82	
Yellow-necked mouse (*Apodemus flavicollis*)	1.39	6.51	17.59	
Habitat use of wood mice after radio tracking				
Proportion of habitat types to home range (MCP), based on 15 individuals tracked for one night (whole observed period) [mean] (90%ile)	Winter cereal fields		93.27 %	(100.0)
	Meadow / grass stripe		4.72 %	(6.80)
	Hedgerow / shrub		1.36 %	(4.85)
	Other habitats (track, ditch)		0.64 %	(1.86)

Portion of time (PT) in habitat of wood mice after radio tracking			
potential foraging time (surface activity only) spent in winter cereal fields; based on 15 individuals, [mean], (90%tile)	BBCH group 1 (p.g.s.*0-2) n = 0	Not conducted (1)	
	BBCH group 2 (p.g.s.*3-6) n = 12	88.31 %	(100.00)
	BBCH group 3 (p.g.s. * 7-9) n = 3	89.31 %	(99.43)
	Total	88.51 %	(100.00)
Habitat use of common voles after radio tracking			
Proportion of habitat types to home range (MCP), based on 18 individuals (19 tracking sessions) tracked for 24 h [mean], (90%ile)	Winter cereal fields	89.94 %	(100.00)
	Meadow/ grass stripe	8.58 %	(100.00)
	Hedgerow / shrub	1.48 %	(100.00)
Portion of time (PT) in habitat of common voles after radio tracking			
potential foraging time (whole observed period) spent in winter cereal fields; based on 19 tracking sessions, [mean], (90%ile)	BBCH group 1 (p.g.s.*0-2) n = 0	Not conducted (1)	
	BBCH group 2 (p.g.s.*3-6) n = 8	63.52 %	(100.00)
	BBCH group 3 (p.g.s. * 7-9) n = 11	93.78 %	(100.00)
	Total	83.86 %	(100.00)

* p.g.s. abbreviation for principle growth stage

(1) No radio-tracking conducted; due to late authorisation, however, no common voles and only very few wood mice could be trapped on the filed prior to BBCH group 2 (p.g.s. 3 - 6)

Small mammal trapping

During the whole study period the trapping effort summed up to 20,224 trap nights on all study plots. From 4th May until the end of the study (period when individual marking of all species was permitted), 424 known individuals of 6 species were captured within 8,576 trapnights. According to the different number of traps the trapping effort in the fields was three times as high as in the surrounding. In order to get a comparable measure for both types of habitat, the trapping efficiency was calculated, which is expressed by the number of captures per 100 trapnights. A comparison of trapping efficiencies of a species indicates in which habitat this species is more likely to be captured. The highest trapping efficiency by far was shown by bank voles in traps set up in the surrounding vegetation (16.93 captures per 100 trapnights). In comparison values for wood mice and common voles were much lower (surrounding: 1.17 captures/100 tn and 0.89 captures/100 tn respectively). Apart from plot 1 both species were more often captured in the field traps than in the surrounding traps. This was more obvious for the common vole with a trapping efficiency 5 times as high in the field than in the surrounding (no. of captures per 100 trap nights: field: 4.67, surrounding: 0.89) than for the wood mouse where it was only twice as high (no. of captures per 100 trap nights: field: 2.51, surrounding: 1.17). These results suggest a higher density of common voles in the field compared to the off-field habitat. Trapping efficiency for yellow-necked mice (*Apodemus flavicollis*) and Field voles (*Microtus agrestis*) was much higher in the surroundings than in the field.

Trapping efficiency was additionally calculated for periods of different crop stages (according to BBCH group Table 1) separately for each study plot. On plots 1-3 the trapping efficiency for wood mice inside the cereal field increased clearly in relation to trappings in the surrounding, with proceeding crop stages. On plot 1 however both wood mice and common voles were in the beginning of the investigation (BBCH p.g.s.2) much more often captured in the surrounding traps and at BBCH 3-6 the trapping efficiency for field and surrounding was approximately equal. On plot 4 both study species were exclusively trapped in the field traps; the same accounts for the common voles on plot 3.

Therefore, the vast increase in trapping efficiency from BBCH group 2 to 3 in common voles on plots 3 and 4 simply reflects the great increase in abundance of this species. On plots 1 and 2 however,

common voles were trapped in field- as well as in off-field traps. Here the trapping efficiency of common voles inside the field increased in relation to the trappings off-field.

Radio tracking and tracking data compilation

The radio tracking data of 18 common voles and 15 wood mice were used to calculate home range sizes and the portion of time individuals spent potentially foraging in the winter cereal fields and other surrounding habitats. Each individual common vole was continuously tracked for one 24 h period and wood mice were tracked from dusk till dawn. One female wood mouse (no. 15, ID: 7200500077) was tracked for 24 h, but the animal's activity was restricted to night time. This was in compliance with literature about activity pattern of wood mouse. Based on these findings it was decided to track the other wood mice only during their periods of activity, *i.e.* at night. The first tracking session of wood mouse number 3 (ID: 7200502562; 2005-05-06/07) was discarded because the unknown time was too great (the signal was lost for some time during tracking and the time the animal left its nest is unknown). In five wood mice (animals no. 3, 6, 9, 10 and 11) the potential foraging time is restricted to the observed active period because the animals had already left the nest when it was found by the tracker. Common vole number 7 (ID: 7200443874; 2005-06-03/04) was only tracked for 16:14 h. The telemetry data of this animal were included in analyses but it has to be noted that 100% of the tracking time corresponds to 16:14h instead of 24h.

Feeding observations

Foraging on cereal plants was regularly noticed during radio tracking of common voles by visual and audible observations. Traces of gnawed stems and spikes of cereal plants were also found in burrow entrances of common voles. At earlier crop stages (BBCH principal growth stage up to 6) common voles seemed to prefer the juicy lower parts of the stems, which were regularly discovered in the holes. With progressing development of the fruit stages spikes and dehusked seeds but less stems were found in the burrow entrances of common voles. Other remains of foraged cereal plants could not readily associated with a certain rodent species. Some of these findings were documented by photographs.

Discussion and Evaluation

In order to assess the risk of plant protecting products to wild small mammals in a realistic manner, knowledge on the species distribution, their habitat use and behaviour is necessary. Field observations in and around fields of the concerned crop can reliably provide these information. Small mammals, primarily rodents, due to their low body mass and their relatively small home ranges, may be more at risk than larger vertebrates.

Evaluation

The results for live trapping as well as for radio tracking confirmed that the wood mouse and the common vole are the most relevant small mammal species to be of concern regarding the application of pesticides in winter cereal crops during spring and summer.

Radio tracking of 15 individual wood mice and 18 common voles in winter cereal fields and adjacent habitats in the western part of Sachsen-Anhalt showed that this crop was used as a main feeding and nesting habitat by these species.

More than half (60%) of the tracked wood mice spent 100% of their potential foraging time (known active period) in the cereal field habitat, and 8 of these 9 individuals also had their nests inside the field. In common voles 10 out of 18 individuals spent 100 % of the potential foraging time (24h) in the cereal field, while one individual did not use the field as feeding habitat at all. In both wood mice and common voles there was a significant difference in the mean PTvalues for cereal fields between BBCH groups 2 and 3. The radio tracked individuals spent more time in the winter cereal fields when the crop stages were more developed.

In wood mice the location of the nest site inside the field appears to positively influence the extent of cereal habitat use (nest sites in common voles could not be determined in most cases). The significance

of winter cereal fields as a feeding and nesting habitat for both species is also supported by the live trapping data of the population of these species within the study area.

Together with the yellow-necked mouse, both the wood mouse and the common vole were the species most likely to be captured in the cereal field. There was a noticeable increase in trapping efficiency of both wood mice and common voles with progressing crop stages. The attractiveness for the cereal field habitat may have grown for common voles with increasing crop height providing more cover and a more diverse food supply which was offered by emerging herbaceous plants within the crop in summer for this mainly folivorous species. For the wood mouse as a facultative granivorous species the spikes of the cereal plants represent an attractive food source. However, wood mice may have also sought the field habitat in order to avoid competition with the congeneric yellow-necked mouse within the surrounding habitat.

For risk assessment purpose values for the portion of time spent foraging in the cereal fields (PT) were calculated from the radio tracking data: On average wood mice spent 63.52% and 93.78% of their potential foraging time in cereal fields of BBCH principal growth stages 3-6 and 7-9, respectively. The mean potential foraging time in common voles accounted for 63.52% in BBCH principal growth stages 3-6, and for 93.78% in cereal fields with BBCH stages 7-9, respectively.

The PT values indicate the cereal field habitat offered a significant but not exclusive feeding habitat for wood mice and common voles. However, at early growth stages the use of the fields is much lower than at later growth stages.

III. Conclusion

The observations confirmed that wood mice and common voles were the most relevant species and most likely to be captured in cereal fields during spring and summer.

Both species were present on all study plots; however, the abundance and appearance differed between the plots. This first appearance of common voles on the field was observed beginning of April (BBCH principle growth stage 3, crop height ≥ 15 cm). The wood mouse was present from the beginning of the investigation period in March (BBCH principle growth stage 2). Apart from one trapping plot (4) wood mice were regularly captured, yet populations showed a slight decrease in summer (BBCH principle growth stages 7-8). Common voles were not trapped on the fields before beginning of April.

In more developed growth stages of the crop (BBCH principle growth stage 3 and later), radio tracking of 15 individual wood mice and 18 common voles in winter cereal fields and adjacent habitats showed that this crop was used as a main feeding and nesting habitat by these species.

More than half (60%) of the tracked wood mice spent 100% of their potential foraging time (known active period) in the cereal field habitat. In common voles 10 out of 18 individuals spent 100 % of the potential foraging time (24h) in the cereal field, while one individual did not use the field as feeding habitat whilst being radio-tracked. The significance of winter cereal fields as a feeding and nesting habitat for both species is also supported by the live-trapping data of the population of these species within the study area.

For risk assessment purposes the portion of time spent potentially foraging in the cereal fields (PT) was calculated from the radio tracking data according to the principle crop growth stages defined. Wood mice spent more than 50% of their potential foraging time in the cereal field already at BBCH principal growth stage 3, whilst typically common voles did not spent significant foraging time on the field before BBCH principal growth stages 4 or 5.

Over the whole observation period, based on the minimum convex polygon the cereal field habitat accounted in average for approximately 90% of the home ranges of the radio tracked wood mice and common voles. Both the PT values and the proportions of habitat use indicate that during late spring and early summer the cereal field habitat offered a significant but not exclusive feeding habitat for all tracked wood mice and common voles.

Assessment and conclusion by applicant:

This ecological monitoring study was conducted to GLP but was not conducted according to a specific test guideline. However, this is typical of studies of this type therefore the study is still considered to be acceptable for regulatory purposes by supporting various aspects of the Bird & Mammal risk assessment.

The results of this study have been used as part of the refined risk assessment of the small herbivorous mammal "vole" scenario, specifically to support the use of the common vole and the wood mouse as a suitable focal species in cereal fields.

The observations in the study confirmed that wood mice and common voles were the most relevant species and most likely to be captured in cereal fields during spring and summer. Thus, the common vole and the wood mouse are considered to be suitable focal species for the refined risk assessment of small herbivorous mammals.

For the common vole a mean PT of 0.635 was determined for cereals at principal growth stages 3 – 6. However, it is noted that the 90th percentile PT value was 1.0 therefore a PT of 1.0 has been used in the refined risk assessments.

Data Point:	KCP 10.1.2.2/03
Report Author:	[redacted]
Report Year:	2019
Report Title:	Relevance of body weight effects for the population development of common voles and its significance in regulatory risk assessment of pesticides in the European Union
Report No:	M-669216-01-1
Document No:	M-669216-01-1
Guideline(s) followed in study:	None
Deviations from current test guideline:	None
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	not applicable
Acceptability/Reliability:	Yes

I. Background

The common vole (*Microtus arvalis)* is typically the wild mammal species driving regulatory pesticide risk assessment (RA) in Europe. The risk assessment endpoint for wild mammals is taken from the studies conducted mainly with rodents for the toxicological part of the dossier. Body weight effects in these studies are often driving the selection of the No Observed Adverse Effect Level (NOAEL) used for wildlife risk assessment. Thus, assessing body weight effects in voles very frequently constitutes a key scenario in the RA. Although many studies on ecology, reproductive biology, population genetics, and other aspects of common voles are available, the relevance of body weight for their survival and reproduction has not yet been specifically analysed. There is also little guidance on how to quantitatively deal with body weight effects in the regulatory risk assessment of pesticides.

II. Results

We evaluated the population relevance of body weight effects on voles by analysis of a dataset from multi-annual study with repeated life-trapping and genotyping, and have correlated body weight with reproductive success, taking account of the seasonality of body weight. Body weight and growth were similar between reproducing and non-reproducing females. The number of confirmed offspring indicated no correlation with parental body weight. Reproductive success of the voles was mainly

influenced by the date of birth, *i.e.*, animals born in Spring have a higher chance to reproduce. Body weight did not correlate with life span during most of the year, except for autumn. Animals weighing < 15 g in October did not survive winter.

III. Discussion

In toxicology studies such as the rat reproduction study, in which animals are exposed to treated diet for several months, effects on body weight are frequently observed (most often in form of retarded growth, rather than actual body weight loss). Since the results from these studies are used for the wild mammal risk assessment of pesticides, the question arises to what extent effects on body weight may affect populations of free ranging animals exposed to pesticides under field conditions.

Already EFSA noted that there were "no quantitative experimental data to define the level of body weight change that is associated with impaired mating performance or parental care". However, no more guidance was then given in the relevant EFSA guidance on how to quantitatively interpret body weight effects observed in the laboratory or how to translate these to field conditions for risk assessment.

The generic focal species scenario "small herbivorous mammals", *i.e.*, Common voles, often drives the initial steps of pesticide RA in the EU. To date, the relevance of body weight on free ranging common voles has never been studied with regard to pesticides, although body weight is typically measured during capture–mark–recapture field effect studies.

The present evaluation is based on a unique dataset from a live trapping study conducted over 3 years and from genotyping of more than a thousand individual voles. Since animals were kept in outdoor enclosures from which they could not move away, the likelihood of trapping them was high. Therefore, information on their life span is considered robust. Since not all animals could be genotyped due to practical reasons, it is possible that some offspring were not detected. However, since a relatively large number of 1255 individuals were genotyped, the data can be considered adequate to address the objective.

A first remarkable result of the present analysis was that about 80% of all females and about 90% of all males had no genetically confirmed offspring. Hence, a considerable proportion of the population did not reproduce or did not reproduce successfully, while relatively few animals produced most offspring (females and males produced up to 13 and 32 pups, respectively).

This means that even under the optimal conditions of this study (grassland habitat, ground predators excluded, population density was in a normal range), the availability of home ranges was a limiting factor for the population. In turn, 80% and 90% of non-reproducing females and males, respectively, provided a considerable 'reproductive reserve', which could start to reproduce when becoming resident, or when reproducing animals disappeared (e.g., by emigration, predation or agricultural practice). This population resilience is not only relevant for pesticide risk assessment but also explains why common vole populations recover very quickly after rodenticide application. Although voles have short lifespans (during the breeding season most animals live only about a month), they exhibit a high reproductive output and often disperse from their natal areas.

Body weight effects, as observed in toxicological studies, could potential impact reproductive success of voles, for example during the "breeding phases" defined by EFSA: "Establish breeding site", "pairing" and "mating". For example, smaller female voles may potentially have a lower reproductive success due to competition over breeding territories.

However, females of small mammal species in agricultural fields are not very selective regarding mates, and males, which have larger home ranges encompassing a number of female home ranges, are only loosely connected with females and thus mate with a large number of females (*i.e.*, polygynous mating system with multiple paternity). Multiple paternity or polyandry is seen as a common female strategy to increase genetic diversity of offspring or to avoid infanticide. Multiple paternities within a litter have been described in common voles, several other small mammals and animals in general. Thus, the actual mating system of common voles is in fact very resilient to effects on individuals and this may also

explain that differences in adult body weight of factor 2 or more were not associated in our study with measurable differences in reproductive success, a key element of the regulatory protection goal.

The focus of the present evaluation was to determine to what extent body weight had an effect on reproduction and survival, to inform the risk assessment or management of small mammals.

However, since common vole body weight showed a typical seasonal trend as previously reported, seasonality needed to be taken into account. The main factor influencing reproductive success (measured as the number of genetically confirmed offspring) was the month of birth. Females born early in the season had more offspring than females born later in the season. Body weights were generally higher early in the season (before population density reaches its peak) than in late summer or later. Hence, one might suspect that body weight is related to a higher number of offspring. However, this is not the case, because when seasonality was accounted for by comparing the number of confirmed offspring and female bodyweight month by month, it was found that larger body weight did not relate to more offspring. That is, for each month, there was no correlation between body weight and the number of confirmed offspring. Overall, this means that seasonally changing body weight does not seem to affect reproductive success, but that the single most influential factor affecting it is the time of birth (or in other words, the time animals have for reproduction). Also, when comparing the body weights of successfully reproducing females (i.e. those with confirmed offspring) and unsuccessfully reproducing females (*i.e.*, those without confirmed offspring), no influence of body weight was found.

In contrast, regarding life span, an effect of body weight was found in young animals born late in the year: when comparing life span and body weight month by month (again to take account of seasonality), it was found that of all animals caught the first time in autumn (October), only those with a body weight of at least 15 g survived until the next year. However, survival of animals caught the first time in October was generally low (only 13% survived until the next year). But a low winter survival of animals born late in the season probably does not affect populations much, since only about 15% of all 'first captures' were caught in October or later.

Before October, body weight and survival did not correlate. In this context, it is interesting to see that body weight is generally highest in late spring and summer when survival is typically lowest. These results are in line with findings in bank voles by Koskela who studied the impact of litter size manipulation in outdoor enclosures in Finland. An artificial increase of litter size related to a lower body weight at weaning and a reduction of litter size resulted in larger weaning weights. While litter size manipulation had no effect on winter survival, survival of pups during lactation was reduced for enlarged litters. However, a higher female weaning weight related to a slightly higher winter survival, independent of litter size manipulation (survival of male offspring was not analysed). Adult female weight did not, however, explain the probability of surviving over winter.

IV. Conclusions

These results demonstrate no detectable influence of common vole body weight on reproductive success and survival during most times of the year. The results of this study suggest that, additional to the hazard information from toxicity studies, ecological information on voles as a typical species of concern should be considered in the regulatory risk assessment of pesticides.

Assessment and conclusion by applicant:

This summary relates to a literature paper which has been written to highlight that the body weight of the common vole would not appear to affect the reproductive success of this species. The study revealed that there was no detectable influence of common vole body weight on the reproductive success and survival during most time of the year and that reproductive success was mainly influenced by the date of birth.

The paper has been referred to in the mammalian risk assessment to support the notion that the relatively small reductions in body weight recorded in the rat two-generation study will not have an adverse effect at the population level and are therefore not ecotoxicologically relevant.

Data Point:	KCP 10.1.2.2/04
Report Author:	[redacted]
Report Year:	2013
Report Title:	Common vole (Microtus arvalis) ecology and management: implications for risk assessment of plant protection products
Report No:	M-476622-01-1
Document No:	M-476622-01-1
Guideline(s) followed in study:	not applicable
Deviations from current test guideline:	None
Previous evaluation:	No, not previously submitted -
GLP/Officially recognised testing facilities:	not applicable
Acceptability/Reliability:	Yes

Abstract

Common voles (*Microtus arvalis*) are common small mammals in some European landscapes. They can be a major rodent pest in European agriculture and they are also representative generic focal small herbivorous mammal species used in risk assessment for plant protection products. In this paper, common vole population dynamics, habitat and food preferences, pest potential and use of the common vole as a model small wild mammal species in the risk assessment process were reviewed. Common voles are a component of agroecosystems in many parts in many parts of Europe, inhabiting agricultural areas (secondary habitats) when the carrying capacity of primary grassland habitats is exceeded. Colonisation of secondary habitats occurs during multiannual outbreaks, when population sizes can exceed 1000 individuals ha^{-1}. In such cases, in-crop common vole population control management has been practised to avoid significant crop damage. The species' status as a crop pest, high fecundity, resilience to disturbance and intermittent colonisation of crop habitats are important characteristics that should be reflected in risk assessment. Based on the information provided in the scientific literature, it seems justified to modify elements of the current risk assessment scheme for plant protection products, including the use of realistic food intake rates, reduced assessment factors of the use of alternative focal rodent species in particular European regions. Some of these adjustments are already being applied in some EU member states. Therefore, it seems reasonably consistently to apply such pragmatic and realistic approaches in risk assessment for plant protection products across the EU.

I. Introduction

Agriculture provides food for more than six billion people globally, with agricultural production greatly increased owing to intensification of farming practices such as increased fertiliser applications, improved plant breeding techniques, irrigation, mechanisation and an increased used of plant protection products.

Plant protection products minimise pre- and post-harvest losses in many crops, including grains, vegetables and corn, but also in horticulture and forestry, by regulating plant disease and reducing the impact of invertebrates, weeds and occasionally vertebrates.

The use of plant protection products and their active ingredients is regulated at EU level and nationally at member state level to ensure that products are effective in managing crop pests and safe for humans and the environment. In the regulatory process, pesticide risk is assessed for formulated products and their active ingredients on the basis of scientific studies performed using recognised test procedures with resulting endpoints applied in the risk assessment models. Only active ingredients and formulated

products satisfying the requirements of the risk assessment to protect non-target organisms from effects associated with the application of the plant protection products can be registered for use.

Risk assessment approaches for wild mammals aim to evaluate the potential impact of a pesticide application on a model "representative" species that is likely to be present in the crop at the time of application. Typically, a model species will have high food intake rate (FIR), consume mostly a relevant type of food (*i.e.* a food type potentially carrying residues) and have a low body weight, all of which maximises the potential exposure to and risk from the pesticide. Under the current scheme of the mammalian risk assessment, the common vole is such a model species representing herbivorous mammals. In agroecosystems, the common vole is an important component of the food web, providing ecosystem can provide shelter for many other species.

However, the common vole is also important vertebrate pest species in many crop types across the European agricultural landscapes. It consumes plant material (*e.g.* leaves, stems, seeds, roots, bark) from several agricultural, horticultural and forestry plants, which can result in significant crop damage. Outbreaks of common voles occur every 2 – 5 years. During outbreaks, farmers typically manage the associated damage by applying rodenticides directly to tunnel entrances. Where possible, farmers can use indirect control methods to manage common voles, such as decreasing vegetation height and cover, which removes food and also reduces shelter from predation.

Within the framework of commission regulation 1107/2009 for placing of plant protection products on the market within the EU, mammalian environmental risk assessments are performed according to guidance presented in an EFSA (2009) guidance document. Within this guidance, the common vole is the representative generic focal small herbivorous mammal species used in the acute and chronic risk assessments, considered relevant for almost all crop types. With a low body weight and a high food intake rate, the common vole has a high potential exposure in crops following product application.

The uncertainty as to how to deal with common voles in risk assessment has remained a constant feature of small herbivorous mammal risk assessment under EFSA (2009) guidance.

This article presents a review of common vole population dynamics, biology and behaviour, including habitat preferences and crop damage potential relevant to risk assessment. In the review, refined approaches to the use of common voles in the risk assessment of plant protection products within the EU regulatory framework on the basis of realistic and scientifically based information are discussed.

II. Discussion

When considering voles in risk assessment, a realistic position on the importance of voles to the agroecosystem should be taken. Published information highlights opportunities to balance risk assessment with characteristics of common vole biology and ecology and pest status.

Habitat preferences

Common voles are essential food web components ensuring energy flow through the trophic levels as a significant primary consumer. They are an important food source for predators within the food chain; for example, raptor species adapt their abundance to coincide with outbreaks of small mammals. This has obvious ecological benefits, but can also result in conservation issues during times of common vole population decline when large predator populations search for alternative food. Common voles are an important pest species in multiple crops, causing significant levels of damage. Their distribution and damage potential is widespread across agricultural landscapes within Europe. Common vole populations peak usually seasonally during autumn. Multiannually, there is a long-term pattern of vole population growth and decline that results in outbreaks occurring in about 2–5 year periods, which means that naturally occurring seasonal and multiannual fluctuations are the rule for common vole populations. The preferred primary habitat of common voles is steppe, which comprises grassland, pasture and meadow with mixed grassland, herbs and weeds that provide appropriate cover to avoid predation. For common voles, many cropped areas are considered to be secondary habitats, and significant invasion into them occurs when there is a population outbreak. In contrast to primary habitats, these secondary habitats cannot maintain common vole populations sustainably for long periods owing to the seasonal nature of

farming, where populations are regularly disrupted by harvest and tilling. Although the common vole is indicated as the representative generic focal species in screening and tier 1 risk assessments under EFSA (2009), population dynamics and habitat preferences indicate that in the period between population outbreaks the likelihood of significant numbers of common voles being found in secondary habitats such as grain crops, vegetables and sugar beet is low. During vole population outbreaks, the density of voles in primary habitats is high, which is likely to provide a considerable buffer for potential adverse effects of plant protection products on common vole populations in secondary habitats such as cropped areas. Inclusion of different levels of comparative risk in primary and secondary habitats for a pest such as the common vole is considered to be appropriate to ensure a sufficient population density is maintained in the primary habitat. This contributes to maintaining the protection goal to avoid long-term detrimental effects on common vole populations.

Managing common vole populations

In Europe, few rodenticidal compounds are used regularly for direct control of common vole populations in crop habitats. The use of rodenticides and alternative methods can reduce crop damage. However, even with such extensive direct action during outbreaks, *Microtus* populations are seen to recover relatively quickly following rodenticide application, although no data are available for common voles. These findings, along with the exceptional reproductive potential of common voles, indicate that common voles are anticipated to overcome potential adverse effects of in-crop application of plant protection products at the landscape level.

Use of the common vole in risk assessment

Based on pest status, population dynamics, habitat preference, resilience and the reproductive potential of the common vole, its relevance to environmental risk assessments must be practically established to ensure that, at tier I of the risk assessment process, the risk to voles across multiple crops is realistically assessed. Risk assessment parameters for default generic focal species as defined in Appendix A of the EFSA (2009) bird and mammal guidance document do not always appear to concur with results of scientific observations in field and laboratory studies. For example, EFSA (2009) use of energy balance models indicates that a 25 g vole must consume 1.33 times its own body weight [the default food intake rate/body weight (FIR/bw)] to satisfy the theoretical daily energy expenditure (DEE). However, in laboratory studies, common voles have been found only to consume about a third of their body weight per day, and values as low as 10% based on the uptake of dry matter have been reported. As shown in laboratory studies, even at low temperatures, when food uptake is highest, an amount of food equivalent to about 50% of the body weight is eaten, although this was not verified under field conditions. Re-evaluating certain generic focal species food intake rates that are not in agreement with literature values is an area of future research that, coupled with additional research, could provide realistic food consumption data for use in risk assessment. The common vole is a model species that exists in cropped areas, and, given body weight and food intake rates, represents a worst-case exposure model. It therefore seems reasonable to consider an adjustment to Annex VI (trigger value) to account for the reduced uncertainty associated with the evaluation of derived TER values from acute and reproduction dietary risk assessments. This reduction could follow the model used in Germany, where lower TER trigger values (≥5 in the acute and ≥2 in the chronic risk assessment) are applied for common voles and wood mice. German regulators consider these species to be the worst case exposure models and not simply representatives of the worst-case exposure model. They also stress that mammalian toxicity endpoints are usually derived from studies with laboratory Norway rats (*Rattus norvegicus*) or house mice (*Mus musculus*) which have a close phylogenetic relationship to field rodent species, thereby reducing the interspecies uncertainty associated with extrapolating laboratory endpoints to wild mammals. Thus, adjusting the acute and chronic TER trigger points (as is the case in Germany) would be a realistic and pragmatic approach appropriate across all EU member states. The use of alternative focal species within the same feeding guild (*e.g.* field vole) is a pragmatic approach to risk assessment proposed for the Northern zone where common voles are not widely distributed. However, this position, although pragmatic, cannot be consistently applied across member states. More information is necessary for better assessment of resilience and recovery in common vole populations and for further development and

validation of modelling approaches that can be valuable in assisting decision-making in risk assessment. This information could be obtained from rodent control programmes and field monitoring data evaluating impacts on populations at the agroecosystem level. This information could be used to establish more accurate exposure estimates and to gain a better understanding of the differences in the dynamics of common vole populations when associated with different crop types.

III. Conclusion

Common voles are widely distributed in agroecosystems. The risk of side effects of plant protection products for common voles is limited to individuals present in crops during product application, while populations in off-crop primary habitat refuges remain unaffected. For many crops, the occurrence of common voles is restricted to population outbreaks and is associated with voles becoming significant agricultural pests. Their pest status, highly fluctuating population dynamics, habitat preferences, resilience and high reproductive potential should reduce potential pesticide impact upon common vole populations, but this is not fully reflected in the current risk assessment scheme.

Overall, based on the compelling evidence provided in this document, it is proposed that it would be justified to modify elements of the current risk assessment, for example by refining consumption estimates on the basis of expanded field-collected data on common voles, applying recued TER trigger values universally across all member states and/or advocating alternative focal species where this is considered to be a geographically appropriate. This will ensure that a more realistic and pragmatic approach to wild mammal risk assessment is taken in the assessment of plant protection products.

Assessment and conclusion by applicant:
This literature paper presents arguments for refining several of the assumptions used at Tier I of the EFSA bird & mammal risk assessment for the common vole.

Data Point:	KCP 10.1.2.2/05
Report Author:	[redacted]
Report Year:	2012
Report Title:	Luna Experience - BCS response to the evaluation by the zonal rapporteur member state Greece - Refined risk assessment for small herbivorous mammals and omnivorous birds
Report No:	M-439777-01-1
Document No:	M-439777-01-1
Guideline(s) followed in study:	Regulation (EC) No 1107/2009
Deviations from current test guideline:	None
Previous evaluation:	No, not previously submitted -
GLP/Officially recognised testing facilities:	not applicable
Acceptability/Reliability:	Yes

I. Introduction

In its evaluation of Luna Experience as Rapporteur Member State for the Southern Zone of the EU, Greece concluded that the risk to the Common Vole is not acceptable for certain uses and that “Member States should carefully consider the vole scenarios are relevant and whether they should merit attention or not”.

Such a conclusion would burden other Member States of the Southern Zone with additional work to resolve this question, if it could not be resolved by the Zonal RMS by providing a risk assessment that shows an acceptable risk for these scenarios. This document is intended to demonstrate that voles, even if the scenarios might be unrealistic in Southern Zone Member States, are not at risk by the intended uses of Luna Experience.

Refinement of ecological parameters of the common vole

Tier 1 Risk assessment for the vole as a small herbivorous mammal assumes that the animal only feeds on grasses. This may be true for a short period of time applicable for an acute risk assessment. However, it is not conceivable that voles would eat only grasses over a prolonged period relevant for a long-term risk assessment.

Lüthi *et al*. (2010) investigated diets of voles for various plants in natural habitats (n=98) and in recently sown wild flower fields (n=99) by analyzing stomach contents and faeces samples. The natural habitats were characterized by a prevalence of monocotyledonous plants whereas dicotyledonous plants prevailed in the wild flower fields.

The authors found a preference for monocots in both scenarios, supporting to notion that grasses might be the predominant feed for voles. They also found, however, that despite this preference for monocots, other feed items contributed to a significant part to the total diet. In the setting with dicots dominating the habitat, monocots represented on average 44.3% of the diet, dicots 19.6% and seeds made up for 19.4% (wet weight). The rest were unidentified items. In the natural habitat (monocots predominating), monocots made up for 71% of the diet, dicots represented 11% and seeds 10.7%. Again, the rest were unidentified items.

These findings demonstrate that, even in situations where they could easily satisfy their energy demands by feeding on monocots alone, voles will feed on dicots and other items like seeds and roots too. Therefore the assumption for the long-term risk assessment that voles will feed exclusively on grasses is unrealistically worst-case.

Because of these clear findings and the fact that the study was done within the typical range of distribution of this species the study and its results are deemed highly relevant for introducing more realistic elements into the risk assessment for this species.

II. Conclusions

These findings demonstrate that, even in situations where they could easily satisfy their energy demands by feeding on monocots alone, voles will feed on dicots and other items like seeds and roots too. Therefore the assumption for the long-term risk assessment that voles will feed exclusively on grasses is unrealistically worst-case.

Assessment and conclusion by applicant:

The report presents the results of a review of the diet of the vole from different habitats. The evidence suggests that voles do not feed exclusively on grasses and that other plant material such as dicots and seeds also made up part of the diet. A vole diet of 76.6% grass & cereals, 11.9% non-grass herbs and 11.5% weed seeds has been established.

The information is considered suitable for use in a refined mammalian risk assessment for the vole, however, this has not been used for the risk assessment of Prothioconazole + Spiroxamine EC 460 in cereals.

Data Point:	KCP 10.1.2.2/06
Report Author:	[redacted]
Report Year:	2014
Report Title:	Population modeling · Use of scenarios to avoid different levels of protection
Report No:	M-489393-01-1
Document No:	M-489393-01-1
Guideline(s) followed in study:	not applicable
Deviations from current test guideline:	None
Previous evaluation:	No, not previously submitted -
GLP/Officially recognised testing facilities:	not applicable
Acceptability/Reliability:	Yes

Abstract

The calculation of TER values provides a simple method to obtain an idea of how likely it is to observe effects, given an estimated exposure and toxicity. While ecological and behavioural aspects can be considered in higher tier risk assessments, species specific reproduction or population ecology are not taken into account in TER. We exemplarily show that using the TER results in a different level of protection for different species. However, the use of conservative scenarios for population modelling, developed here for the wood mouse and the common vole, provides a tool to apply the same level of protection in different species.

I. Introduction

TER only takes into account exposure and toxicity. Focussing on the protection goals defined in EFSA (2009) other factors additionally affect the risk on the population level, such as reproduction and population dynamics. This can be demonstrated when comparing recovery of modelled populations of wood mice and common voles in which litter sizes were reduced by 20% in May. While for wood mice a small reduction of population density is visible, no effect is visible in voles.

II. Landscape scenarios

Simulations were conducted in landscapes with varying size, in order to identify a minimum landscape size, which can sustain a "local populations" (a population in classical sense, *e.g.* MacArthur & Wilson, 1967; Wilson, 1971, or population genetic sense, Hardy, 1908, would be much larger). For wood mice landscapes of ~25 ha size were needed, while for common voles 5 ha were sufficient.

To obtain conservative landscape scenarios for population modelling a GIS analysis was conducted calculating a landscape quality measure for all landscapes squares of 50 ha (wood mouse) and 5 ha (common vole) and ranking the resulting millions of landscapes for habitat quality (details in Wang and Luttik, 2013). Simulations were finally conducted with landscapes corresponding to the 10th, 20th, 50th, 80th and 90th percentiles and it was found that wood mice only consistently survived over 20 years in landscapes corresponding to the 10th percentile. This means that wood mice need a relatively large

landscape with a considerable fraction of "good" habitat to survive on the long term, and voles can survive in almost any small landscapes, if there is at least a small fraction of useable habitat available.

Comparison of TER and population level risk

For comparison of TER calculations and population simulations, the following first tier risk assessment was considered as a basis:

Table CP 10.1.2.2/06-1 Body weight (g) for Female Northern Bobwhite Fed KWG 4168 in the Diet for Eight Weeks

Crop/stage[1]	Focal species	NOEL[2] [mg/kg bw]	DDD total [mg/kg bw]	TER_{LT}	Trigger value
Cereals, BBCH ≥ 40	Wood mouse (25% weeds, 50% weed seeds, 25% ground arthropods)	5	0.1563	32.0	5
Cereals, BBCH ≥ 40	Common vole (100% grass)	5	2.8734	1.7	5

[1]AR: 250 g a.s./ha [2]Effect: Reduced litter size

To calculate effects in population simulations it was assumed that the NOEL corresponds to the EC_{10} of a standard dose response curve. Simulations were conducted additionally with higher and lower doses for both species, which result in TER values between 0 and 10 in standard risk assessment.

TER results in a different level of protection for species

Simulations showed that doses which would result in the same TER value in a first tier risk assessment had different effects on the population level in each species. While in voles population level effects were visible only for TER values <1, in wood mice effects were visible for TER values <2-5. This demonstrates that TER results in a different level of protection for the common vole and the wood mouse.

III. Conclusion

The use of TER results in a different level of protection for different species. Conservative, species-specific landscape scenarios developed for population modelling provide a tool to reach the same level of protection in different species.

Assessment and conclusion by applicant:

M-489393-01-1 is a poster presentation summarising some population modelling work which suggests that the TER approach results in a different level of protection for different species with a focus here on the wood mouse and the common vole.

CP 10.1.3 Effects on other terrestrial vertebrate wildlife (reptiles and amphibians)

No data are available with Prothioconazole + Spiroxamine EC 460 for terrestrial vertebrates other than mammals and birds. No additional studies on other terrestrial vertebrates are required in accordance with Commission Regulation (EU) No 283/2013 or 284/2013 and there are currently no risk assessment schemes for reptiles or amphibians.

In the supporting publication by EFSA (2017)[3] to review the biological relevance of the magnitude of effects observed in studies with amphibians and reptiles, it is noted that fish-generated toxicity data seem to be appropriate to cover aquatic amphibians. For terrestrial organisms typically birds and mammals are shown to be more sensitive than amphibians and reptiles to a higher number of substances. Currently data do not allow for extrapolating between groups, however the frequency of cases in which amphibians or reptiles are more sensitive than birds or mammals is around 30%. It can therefore be reasonably assumed therefore that the risk assessment for fish, birds and mammals is likely to be protective of the risk to amphibians and reptiles.

CP 10.2 Effects on aquatic organisms

Toxicity data for spiroxamine and the metabolites of spiroxamine are summarized in the table below. The data include studies previously reviewed and included in the DAR and EFSA conclusion for spiroxamine as well as any previously un-submitted or new studies which have been conducted.

Table CP 10.2-1 Summary of endpoints for toxicity of spiroxamine and metabolites to aquatic organisms

Organism	Test item	Test type	Endpoints		Reference
Fish					
Oncorhynchus mykiss (Rainbow trout)	Spiroxamine	Acute toxicity 96 h (static)	96-hour LC_{50} 18,300 µg a.s./L (mm)	EU	M-006243-01-1
Lepomis macrochirus (Bluegill sunfish)	Spiroxamine	Acute toxicity 96 h (static)	96-hour LC_{50} 7,130 µg a.s./L (mm)	EU	M-006229-01-1
Danio rerio (Zebra fish)	Spiroxamine	Acute toxicity 96 h (static)	**96-hour LC_{50} 2,410 µg a.s./L (mm)**	EU	M-303809-02-1
Oncorhynchus mykiss (Rainbow trout)	Spiroxamine	Chronic toxicity (ELS) 93 d (flow through)	NOEC <62.5 µg a.s./L (nom) (EC_0) 14 µg a.s./L (nom)	EU	M-006232-01-1
		Statistical Re-analysis	EC_{10} >62.5 µg a.s./L (nom)	NEW	M-760407-01-1
Oncorhynchus mykiss (Rainbow trout)	Spiroxamine	Chronic toxicity (ELS, radiolabelled) 96 d (flow through)	NOEC 14.2 µg a.s./L (mm)	EU	M-006449-02-1
		Statistical Re-analysis	EC_{10} 91.5 µg a.s./L (mm) EC_{20} 195 µg a.s./L (mm)	NEW	M-760405-01-1

[3] EFSA supporting publication 2017; EN-1251. Biological relevance of the magnitude of effects (considering mortality, sub-lethal and reproductive effects) observed in studies with amphibians and reptiles in view of population level impacts on amphibians and reptiles.

Organism	Test item	Test type	Endpoints		Reference
Oncorhynchus mykiss (Rainbow trout)	Spiroxamine	Chronic toxicity (ELS; sediment system; pulsed exposure) 56 d	NOEC 3 x 60 µg a.s./L (mm)	EU	M-304369-01-1
Danio rerio (Zebra fish)	Spiroxamine	Chronic toxicity (FFLC) 230 d (flow through)	NOEC 2.6 µg a.s./L (nom)	EU	M-304458-02-1
		Statistical Re-analysis	**EC_{10} 1.88 µg a.s./L (nom)** EC_{20} 4.46 µg a.s./L (nom)	NEW	M-760413-01-1
Danio rerio (Zebra fish)	Spiroxamine	Chronic toxicity (FFLC; sediment system; pulsed exposure) 56 d	EC_{10} (survival) 23.3 µg a.s./L (im) NOEC (biomarker VTG) 15.8 µg a.s./L (im)	EU	M-467979-03-1
		Statistical Re-analysis	EC_{10} not determinable	NEW	M-760412-01-1
Pimephales promelas (Fathead minnow)	Spiroxamine	Fish screening assay	Growth and fertility not affected at up to and including 58.8 µg a.s./L (mm) No effects on endocrine specific biomarker endpoints at up to and including 18.9 µg a.s./L (mm)	EU	M-304833-01-1
Lepomis macrochirus (Bluegill sunfish)	Spiroxamine	BCF	**$BCF_{(whole\ fish)}$ 87** CT_{50} 13 - 19 hours	EU	M-006479-01-1
Aquatic invertebrates					
Daphnia magna	Spiroxamine	Acute toxicity 48 h (static)	48-hour EC_{50} 6,100 µg a.s./L (im)	EU	M-006245-01-1
Daphnia magna	Spiroxamine	Acute toxicity 48 h (radiolabelled; static)	48-hour EC_{50} 6,800 µg a.s./L (mm)	EU	M-006476-01-1
Daphnia magna	Spiroxamine	Acute toxicity 48 h (radiolabelled; flow-through)	**48-hour EC_{50} 3,000 µg a.s./L (mm)**	EU	M-006523-01-1

Organism	Test item	Test type	Endpoints		Reference
Daphnia magna	KWG 4168-N-oxide (M03)	Acute toxicity 48 h (static)	**48-hour EC_{50} >100,000 µg/L (nom)**	EU	M-006702-01-1
Daphnia magna	Spiroxamine	Chronic toxicity 21 d (static-renewal)	NOEC 100 µg a.s./L (nom)	EU	M-006401-01-1
		Statistical Re-analysis	EC_{10} 120 µg a.s./L (nom) EC_{20} 200 µg a.s./L (nom)	NEW	M-761546-01-1
Daphnia magna	Spiroxamine	Chronic toxicity 21 d (radiolabeled; flow-through)	**NOEC 34 µg a.s./L (mm)**	EU	M-006555-01-1
		Statistical Re-analysis	EC_{10} 32 µg a.s./L (mm)* EC_{20} 68 µg a.s./L (mm)*	NEW	M-760409-01-1
Daphnia magna	Spiroxamine	Chronic toxicity 21 d (radiolabeled; static-renewal)	NOEC 47 µg a.s./L (mm)	EU	M-006466-01-1
		Statistical Re-analysis	EC_{10} 39 µg a.s./L (mm) EC_{20} 65 µg a.s./L (mm)	NEW	M-761544-01-1
Aquatic algae and invertebrates	Spiroxamine EC 500	Outdoor mesocosm	Class 1 effects: 1.0 µg a.s./L Class 2 effects: 2.1 µg a.s./L Class 3A effects: 9.3 µg a.s./L **ETO-RAC 0.5 µg a.s./L** ERO-RAC 3.1 µg a.s./L	EU	M-304557-01-1
				NEW	M-690576-01-1
Sediment-dwelling organisms					
Chironomus riparius	Spiroxamine	Chronic toxicity 28 d (static; radiolabelled)	EC_{15} (development time) ~5,600 µg a.s./L (nom) **NOEC (emergence) 5,600 µg a.s./L (nom)**	EU	M-006549-01-1

Organism	Test item	Test type	Endpoints		Reference
		Statistical Re-analysis	EC_{10}/EC_{20} not determinable	NEW	M-760403-01-1
Lumbriculus variegatus	Spiroxamine	Chronic toxicity 28 d (static)	**EC_{10} 7,120 µg a.s./kg sediment (mm)** NOEC 16,700 µg a.s./kg sediment (mm)	NEW	M-688127-01-1
Amphibia					
Xenopus laevis	Spiroxamine	XETA	No indication of endocrine activity on the thyroid axis concluded. A statistically significant increase in flouresence was observed at the 1.6 mg/L treatment but this concentration was above the MTC	NEW	M-762327-01-1
Algae					
Scenedesmus subspicatus	Spiroxamine	72 h (static)	**E_rC_{50} 12 µg a.s./L (mm)** E_bC_{50} 3.2 µg a.s./L (mm)	EU	M-006228-01-1
		Statistical Re-analysis	E_rC_{10} 1.56 µg a.s./L (mm) E_rC_{20} 3.51 µg a.s./L (mm) E_rC_{50} 11.9 µg a.s./L (mm) E_yC_{10} 0.84 µg a.s./L (mm) E_yC_{20} 1.44 µg a.s./L (mm) E_yC_{50} 3.28 µg a.s./L (mm)	NEW	M-761401-01-1
Pseudokirchneriella subcapitata	Spiroxamine	120 h (static)	E_rC_{50} 19.43 µg a.s./L (nom) E_bC_{50} 5.42 µg a.s./L (nom)	EU	M-006518-01-1

Organism	Test item	Test type	Endpoints		Reference
		Statistical Re-analysis	E_rC_{10} 9.20 µg a.s./L (nom) E_rC_{20} 10.9 µg a.s./L (nom) E_rC_{50} 15.2 µg a.s./L (nom) E_yC_{10} 3.60 µg a.s./L (nom) E_yC_{20} 4.73 µg a.s./L (nom) E_yC_{50} 7.99 µg a.s./L (nom)	NEW	M-761402-01-1
Pseudokirchneriella subcapitata	Spiroxamine	96 h (static)	E_rC_{50} >8.14 µg a.s./L (im) E_bC_{50} 5.5 µg a.s./L (im) EC_{50} (cell density) 5.7 µg a.s./L (im)	EU	M-006533-01-1
		Statistical Re-analysis	E_rC_{10} 4.93 µg a.s./L (im) E_rC_{20} 10.5 µg a.s./L (im) E_rC_{50} >8.14 µg a.s./L (im) E_yC_{10} 1.29 µg a.s./L (im) E_yC_{20} 2.18 µg a.s./L (im) E_yC_{50} 5.90 µg a.s./L (im)	NEW	M-761427-01-1
Desmodesmus subspicatus	Spiroxamine	72 h (static)	E_rC_{10} 9.53 µg a.s./L (nom) E_rC_{20} 11.4 µg a.s./L (nom) E_rC_{50} 175 µg a.s./L (nom)	EU	M-273962-01-1
		Statistical Re-analysis	E_yC_{10} not determinable E_yC_{20} not determinable E_yC_{50} 10.5 µg a.s./L (nom)	NEW	M-761457-01-1
Skeletonema costatum	Spiroxamine	96 h (static)	**E_rC_{50} 6.3 µg a.s./L (im)**	EU	M-006512-01-1

Organism	Test item	Test type	Endpoints		Reference
		Statistical Re-analysis	E_rC_{10} not determinable E_rC_{20} not determinable E_rC_{50} 6.33 µg a.s./L (im) E_yC_{10} not determinable E_yC_{20} not determinable E_yC_{50} 1.29 µg a.s./L (im)	NEW	M-761414-01-1
Anabaena flos-aquae	Spiroxamine	96 h (static)	EC_{50} (cell density) >990 µg a.s./L (mm)	EU	M-006537-01-1
Navicula pelliculosa	Spiroxamine	96 h (static)	E_rC_{50} 11.85 µg a.s./L (mm)	EU	M-006542-01-1
		Statistical Re-analysis	E_rC_{10} 8.36 µg a.s./L (mm) E_rC_{20} 9.44 µg a.s./L (mm) E_rC_{50} 11.9 µg a.s./L (mm)	EU	M-280532-01-1
		Statistical Re-analysis	E_yC_{10} 6.83 µg a.s./L (mm) E_yC_{20} 7.60 µg a.s./L (mm) E_yC_{50} 9.32 µg a.s./L (mm)	NEW	M-761458-01-1
Desmodesmus subspicatus	KWG 4168-desethyl (M01)	72 h (static)	E_rC_{10} not determinable E_rC_{20} 42.9 µg/L (nom) **E_rC_{50} 737 µg/L (nom)**	EU	M-288232-01-1
		Statistical Re-analysis	E_yC_{10} not determinable E_yC_{20} not determinable E_yC_{50} 30.6 µg/L (nom)	NEW	M-761465-01-1

Organism	Test item	Test type	Endpoints		Reference
Pseudokirchneriella subcapitata	KWG 4168-despropyl (M02)	72 h (static)	E_rC_{10} 20.3 µg/L (im) E_rC_{20} 55.7 µg/L (im) **E_rC_{50} 383 µg/L (im)** E_yC_{10} n.d. E_yC_{20} 14.8 µg/L (im) E_yC_{50} 42.5 µg/L (im)	NEW	M-680692-01-2
Desmodesmus subspicatus	KWG 4168-N-oxide (M03)	72 h (static)	E_rC_{10} 658 µg/L (nom) E_rC_{20} 2,500 µg/L (nom) **E_rC_{50} 31,700 µg/L (nom)**	EU	M-288035-01-1
		Statistical Re-analysis	E_yC_{10} 218 µg/L (nom) E_yC_{20} 536 µg/L (nom) E_yC_{50} 2835 µg/L (nom)	NEW	M-761467-01-1
Desmodesmus subspicatus	KWG 4168-acid (M06)	72 h (static)	E_rC_{10} >3,200 µg/L (nom) E_rC_{20} >3,200 µg/L (nom) **E_rC_{50} >3,200 µg/L (nom)**	EU	M-309818-01-1
		Statistical Re-analysis	Not determinable. E_yC_{50} considered to be >3,200 µg/L (nom)	NEW	M-761469-01-1
Aquatic plants					
Lemna gibba	Spiroxamine	14 d (static)	**14-day EC_{50} (frond counts) 1,910 µg a.s./L (mm)** 14-day E_rC_{50} 2,650 µg a.s./L (mm)	EU	M-006497-01-1
		Statistical Re-analysis	frond number 7-day E_rC_{10} 2,060 µg a.s./L (mm) 7-day E_rC_{20} 3,110 µg a.s./L (mm) 7-day E_rC_{50} 6,780 µg a.s./L (mm)	EU	M-303421-01-1

Organism	Test item	Test type	Endpoints		Reference
			frond number 14-day E_rC_{10} 1,260 µg a.s./L (mm) 14-day E_rC_{20} 1,820 µg a.s./L (mm) 14-day E_rC_{50} 3,170 µg a.s./L (mm)		
		Statistical Re-analysis	7-day E_yC_{10} 220 µg a.s./L (mm) 7-day E_yC_{20} 620 µg a.s./L (mm) 7-day E_yC_{50} 3,020 µg a.s./L (mm) 14-day E_yC_{10} 560 µg a.s./L (mm) 14-day E_yC_{20} 930 µg a.s./L (mm) 14-day E_yC_{50} 1,990 µg a.s./L (mm)	NEW	M-760417-01-1
		14 d (static)	14-day EC_{50} (frond number) 2,760 µg a.s./L (mm) 14-day EC_{50} (biomass) 9,380 µg a.s./L (mm)	EU	M-006540-01-1
Lemna gibba	Spiroxamine		frond number 7-day E_rC_{10} 3,510 µg a.s./L (mm) 7-day E_rC_{20} 4,130 µg a.s./L (mm) 7-day E_rC_{50} 5,600 µg a.s./L (mm)		
		Statistical Re-analysis	dry weight 14-day E_rC_{10} 4,760 µg a.s./L (mm) 14-day E_rC_{20} 7,960 µg a.s./L (mm) 14-day E_rC_{50} 21,200 µg a.s./L (mm)	EU	M-303443-01-1

Organism	Test item	Test type	Endpoints		Reference
		Statistical Re-analysis	frond number 14-day E_rC_{10} 2,530 µg a.s./L (mm) 14-day E_rC_{20} 2,790 µg a.s./L (mm) 14-day E_rC_{50} 3,670 µg a.s./L (mm) 7-day E_yC_{10} 2,340 µg a.s./L (mm) 7-day E_yC_{20} 2,860 µg a.s./L (mm) 7-day E_yC_{50} 4,230 µg a.s./L (mm) 14-day E_rC_{10} 1,290 µg a.s./L (mm) 14-day E_rC_{20} 1,770 µg a.s./L (mm) 14-day E_yC_{50} 2,860 µg a.s./L (mm)	NEW	M-760416-01-1

EU: previously evaluated as part of the original EU review and listed in EFSA conclusion and DAR
NEW: new study or data generated since the previous EU review or previously not submitted
Values in **bold** have been used in the risk assessment
mm = Results based on mean measured test concentrations
nom = Results based on nominal test concentrations
im = Results based on initial measured test concentrations
* EC_{10} considered unreliable therefore not used in risk assessment

Toxicity data for prothioconazole and the metabolites of prothioconazole are summarized in the table below.

Table CP 10.2-2 Summary of aquatic toxicity studies with prothioconazole, prothioconazole-desthio, prothioconazole-S-methyl and 1,2,4-Triazole

Organism	Test item	Test type	Endpoints		Reference
Fish					
Oncorhynchus mykiss (Rainbow trout)	Prothioconazole	Acute toxicity	**LC_{50} 1,830 µg a.s./L**	EU	EFSA Conclusion[1]
Lepomis macrochirus (Bluegill sunfish)	Prothioconazole	Acute toxicity	LC_{50} 4,590 µg a.s./L	EU	
Cyprinus carpio (Common carp)	Prothioconazole	Acute toxicity	LC_{50} 6,910 µg a.s./L	EU	

Organism	Test item	Test type	Endpoints		Reference
Oncorhynchus mykiss (Rainbow trout)	Prothioconazole	Chronic toxicity (ELS)	**NOEC 308 µg a.s./L**	EU	
Oncorhynchus mykiss (Rainbow trout)	Prothioconazole-desthio	Acute toxicity	**LC_{50} 6,630 µg/L**	EU	
Leuciscus idus melanotus (Golden orfe)	Prothioconazole-desthio	Acute toxicity	LC_{50} 13,200 µg/L	EU	
Oncorhynchus mykiss (Rainbow trout)	Prothioconazole-desthio	Chronic toxicity (ELS)	**NOEC 3.34 µg/L**	EU	
Oncorhynchus mykiss (Rainbow trout)	Prothioconazole-S-methyl	Acute toxicity	**LC_{50} 1,800 µg/L**	EU	
Oncorhynchus mykiss (Rainbow trout)	1,2,4-Triazole	Acute toxicity	**LC_{50} 498,000 µg/L**	EU	
Oncorhynchus mykiss (Rainbow trout)	1,2,4-Triazole	Chronic toxicity	**NOEC 3,200 µg/L**	EU	
Lepomis macrochirus (Bluegill sunfish)	Prothioconazole	BCF	**BCF_{parent} 19.7** CT_{50} 0.8 days 9% residues after 14 days depuration	EU	
Lepomis macrochirus (Bluegill sunfish)	Prothioconazole-desthio	BCF	**BCF_{parent} 65** CT_{50} 0.4 - 0.5 days 4% residues after 14 days depuration	EU	
Aquatic invertebrates					
Daphnia magna	Prothioconazole	Acute toxicity	**EC_{50} 1,300 µg a.s./L**	EU	EFSA Conclusion[1]
Daphnia magna	Prothioconazole	Chronic toxicity	**NOEC 560 µg a.s./L**	EU	
Daphnia magna	Prothioconazole-desthio	Acute toxicity	**EC_{50} >10,000 µg/L**	EU	
Daphnia magna	Prothioconazole-desthio	Chronic toxicity	**NOEC 100 µg/L**	EU	
Daphnia magna	Prothioconazole-S-methyl	Acute toxicity	**EC_{50} 2,800 µg/L**	EU	
Daphnia magna	1,2,4-Triazole	Acute toxicity	**EC_{50} 900,000 µg/L**	EU	

Organism	Test item	Test type	Endpoints		Reference
Sediment-dwelling organisms					
Chironomus riparius	Prothioconazole	Chronic toxicity	**NOEC 9,140 µg a.s./L**	EU	EFSA Conclusion[1]
Chironomus riparius	Prothioconazole-desthio	Chronic toxicity	**NOEC 2,000 µg/L**	EU	
Algae					
Pseudokirchneriella subcapitata	Prothioconazole	Algal growth inhibition	**E_rC_{50} 2,180 µg a.s./L** E_bC_{50} 1,110 µg a.s./L	EU	
Scenedesmus subspicatus	Prothioconazole-desthio	Algal growth inhibition	**E_rC_{50} 550 µg/L** E_bC_{50} 373 µg/L	EU	
Pseudokirchneriella subcapitata	Prothioconazole-S-methyl	Algal growth inhibition	**E_rC_{50} 47,400 µg/L** E_bC_{50} 3,770 µg/L	EU	
Pseudokirchneriella subcapitata	Prothioconazole-1,2,4-Triazole	Algal growth inhibition	**E_rC_{50} 22,500 µg/L** E_bC_{50} 8,200 µg/L	EU	

EU: previously evaluated as part of the original EU review and listed in EFSA conclusion and DAR
[1] EFSA Scientific Report (2007) 106, 1-98. Conclusion on the peer review of prothioconazole
Values in **bold** have been used in the risk assessment

Toxicity data for Prothioconazole + Spiroxamine EC 460 are summarized in the table below.

Table CP 10.2-3 Summary of endpoints for toxicity of Prothioconazole + Spiroxamine EC 460 to aquatic organisms

Organism	Test item	Test type	Endpoints		Reference
Fish					
Oncorhynchus mykiss (Rainbow trout)	Prothioconazole + Spiroxamine EC 460	Acute toxicity 96 h (static)	**96-hour LC_{50} 6,570 µg/L (nom)** (1,980 µg SPX/L; 1,070 µg PTZ/L)	EU	M-039959-01-1
Daphnia magna	Prothioconazole + Spiroxamine EC 460	Acute toxicity 48 h (static)	**48-hour EC_{50} 6,300 µg/L (nom)** (1,910 µg SPX/L; 995 µg PTZ/L)	EU	M-069578-01-1
Pseudokirchneriella subcapitata	Prothioconazole + Spiroxamine EC 460	72 h (static)	E_rC_{50} 160 µg/L (nom) (47.7 µg SPX/L; 25.8 µg PTZ/L)	EU	M-077013-02-1

Organism	Test item	Test type	Endpoints		Reference
		Statistical Re-analysis	E_rC_{10} not determinable E_rC_{20} 6.34 µg/L (nom) (1.89 µg SPX/L; 1.02 µg PTZ/L) **E_rC_{50} 147 µg/L (nom)** (43.8 µg SPX/L; 23.7 µg PTZ/L) E_yC_{10} not determinable E_yC_{50} not determinable E_rC_{50} 8.46 µg/L (nom) (2.52 µg SPX/L; 1.36 µg PTZ/L)	NEW	M-761433-01-1
Lemna gibba	Prothioconazole + Spiroxamine EC 460	7 d (static renewal)	frond number 7-day E_rC_{10} 8.3 µg/L (nom) 7-day E_rC_{50} 57 µg/L (nom) (17.0 µg SPX/L; 9.18 µg PTZ/L) dry weight 7-day E_yC_{10} 6.3 µg/L (nom) 7-day E_rC_{50} 67 µg/L (nom)	EU	M-077038-01-1

Organism	Test item	Test type	Endpoints		Reference
		Statistical Re-analysis	frond number 7-day E_rC_{10} 7.13 µg/L (nom) 7-day E_rC_{20} 14.3 µg/L (nom) **7-day E_rC_{50} 54.2 µg/L (nom) (16.2 µg SPX/L; 8.73 µg PTZ/L)** 7-day E_yC_{10} 3.93 µg/L (nom) 7-day E_yC_{20} 6.88 µg/L (nom) 7-day E_yC_{50} 20.1 µg/L (nom) (5.99 µg SPX/L; 3.24 µg PTZ/L) dry weight 7-day E_rC_{10} 9.99 µg/L (nom) 7-day E_rC_{20} 40.8 µg/L (nom) 7-day E_rC_{50} 602 µg/L (nom) 7-day E_yC_{10} 3.76 µg/L (nom) 7-day E_yC_{20} 9.24 µg/L (nom) 7-day E_yC_{50} 51.4 µg/L (nom)	NEW	M-760414-01-1

EU: previously evaluated as part of the original EU review and listed in EFSA conclusion and DAR
NEW: new study or data generated since the previous EU review or previously not submitted
Values in **bold** have been used in the risk assessment
nom = Results based on nominal test concentrations

Toxicity endpoints for spiroxamine

For the long-term studies where EC_{10} and EC_{20} values were not already available, these values have been calculated. For each relevant study, a summary of the statistical re-evaluation work immediately follows the summary of the main study. In cases where a valid EC_{10} could be determined, the risk assessment has used the lower value out of the NOEC and the EC_{10}. Furthermore, for the algal and *Lemna* studies where yield had not been determined in the study report, the E_yC_{10}, E_yC_{20} and E_yC_{50} values have been determined, where possible. However, it is noted that the risk assessment has used the growth rate E_rC_{50} values, in accordance with the recommendations of the Aquatic Guidance Document[4].

[4] Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters. EFSA Journal 2013;11(7):3290, 268 pp.

Acute fish data are available using spiroxamine technical for three fish species. The most sensitive species was *Danio rerio* with a 96-hour LC_{50} of 2,410 µg a.s./L. Thus, this endpoint has been used in the acute risk assessment for fish. For chronic fish toxicity there are three fish early life stage studies as well as one standard fish full life cycle study using spiroxamine technical. The lowest endpoint comes from the fish full life cycle study (M-304458-02-1) which gave a NOEC of 2.6 µg a.s./L. In the previous Renewal of Approval for spiroxamine an EC_{10} of 2 µg a.s./L was derived for this study and used in the assessment. EC_x re-evaluation has therefore been conducted for this study and an EC_{10} of 1.88 µg a.s./L has been derived. Thus, the EC_{10} of 1.88 µg a.s./L has been used at Tier I in the chronic fish risk assessment. It is noted that this EC_{10} is below the lowest concentration tested in the study and could therefore be considered unreliable, however, as a precedent for using an EC_{10} for this study has been set in the previous evaluation of spiroxamine the EC_{10} has also been used here. A refined fish full life cycle study using pulsed-exposure and conducted in the presence of sediment (M-467879-03-1) is also available and has been considered as part of a refined risk assessment. This refinement study has been discussed later on in the section.

Three acute *Daphnia* studies are available for spiroxamine technical from which the lowest EC_{50} of 3,000 µg a.s./L was derived. This endpoint has therefore been used in the acute aquatic invertebrate risk assessment. For the chronic aquatic invertebrate risk assessment there are also three *Daphnia* reproduction studies available, the lowest reliable NOEC/EC_{10} being 34 µg a.s./L. A NOEC of 34 µg a.s./L has therefore been used in the chronic aquatic invertebrate risk assessment.

For sediment-dwelling organisms two studies using *Chironomus riparius* are available. No effects were seen at any concentration tested in the formulation study (*i.e.* NOEC ≥2.5 µg a.s./L) therefore the higher NOEC from the technical material study of 5,600 µg a.s./L has been used in the risk assessment for sediment-dwelling organisms. Both studies showed a lack of significant effects therefore it is considered justified to take the highest of the two available NOEC values.

Spiroxamine is a fungicide and therefore, according to the Aquatic Guidance Document, the recommended test species for sediment-dwelling organisms is *Lumbriculus*. A *Lumbriculus* study is available using spiroxamine technical and provides a sediment-based endpoint for use in the risk assessment. The lowest endpoint from this study was an EC_{10} of 7,120 µg a.s./kg sediment. Thus, both the NOEC of 5,600 µg a.s./L from the *Chironomus* study and the EC_{10} of 7,120 µg a.s./kg sediment from the *Lumbriculus* study have been used in the risk assessment for surface water and sediment compartments, respectively.

For the algal risk assessment the lowest bound E_rC_{50} value from the studies with green algal species has been used in the risk assessment (E_rC_{50} of 12 µg a.s./L from study M-006228-01-1). A potentially lower endpoint of >8.14 µg a.s./L is available from study M-006533-01-1 but as this value is not bound (*i.e.* a 'greater than' value) it is considered more appropriate to use the derived E_rC_{50} of 12 µg a.s./L to represent green algae. Spiroxamine is a fungicide therefore additional studies with algal species from a different taxonomic group are not a data requirement, however, several studies are available using algal species which are not green algae, including *Skeletonema costatum, Navicula pelliculosa* and *Anabaena flos-aquae*. The lowest E_rC_{50} from these additional species is for the marine species *Skeletonema costatum* with an E_rC_{50} of 6.3 µg a.s./L. As this value is lower than the E_rC_{50} for the green algal species it is considered necessary to include this in the risk assessment. Thus, both the E_rC_{50} of 12 µg a.s./L and the E_rC_{50} of 6.3 µg a.s./L have been used in the risk assessment to represent freshwater and marine species, respectively.

Spiroxamine is a fungicide therefore data for aquatic macrophytes are not a core data requirement. However, two studies using *Lemna* are available therefore aquatic macrophytes have also been included in the risk assessment. The lowest EC_{50} value was determined to be 1,910 µg a.s./L and has therefore been used in the risk assessment.

A mesocosm study is available using Spiroxamine EC 500 which included zooplankton and algae. Although not conducted with Prothioconazole + Spiroxamine EC 460, the study has been included in the risk assessment here because this study and the endpoint that it provides are considered to be integral

to the risk assessment of spiroxamine. The study has been re-assessed against current requirements including MDD analysis and meets the mimumum requirements. The NOEC based on Class 1 effects was 1.0 µg a.s./L which gives an ETO-RAC of 0.5 µg a.s./L when an assessment factor (AF) of 2 is applied, in accordance with the recommendations of the Aquatic Guidance Document. An ERO-RAC of 3.1 µg a.s./L was also derived based on Class 3A effects at 9.3 µg a.s./L with an AF of 3. For the risk assessment the more conservative ETO-RAC of 0.5 µg a.s./L has been used. The study has not been used as a refinement study in the risk assessment but as the ETO-RAC of 0.5 µg a.s./L is lower than the lowest Tier I algal RAC of 0.63 µg a.s./L, the mesocosm endpoint has been included in the Tier I risk assessment alongside the algal and invertebrate risk assessments.

Metabolites of spiroxamine

For the metabolites there are experimental data available for M01, M02, M03 and M06 with algae. A non-GLP acute *Daphnia* study is available using M03 which has been used in the risk assessment but there are no acute aquatic invertebrate data available for M01, M02 and M06. Furthermore, there are no acute fish data available for any of the metabolites. For the risk assessment it has therefore been necessary to estimate the metabolite toxicity for fish and aquatic invertebrates using the available data with the parent material. It is clear from the algal data that the metabolites are at least one order of magnitude less toxic than spiroxamine (spiroxamine E_rC_{50}: 12 µg a.s./L; M01 E_rC_{50}: 737 µg/L; M02 E_rC_{50}: 383 µg/L; M03 E_rC_{50}: 31,700 µg/L; M06 E_rC_{50}: >3,200 µg/L). The non-GLP acute *Daphnia* study with M03 gave an EC_{50} of >100,000 µg/L which is also much greater than the EC_{50} for spiroxamine of 3,000 µg a.s./L. It is therefore considered justified to use equivalent parent toxicity to represent the metabolites in cases where there are no experimentally determined values. This approach is still considered to be conservative given that the available data with the most sensitive organism group, algae, confirms that the metabolites are at least ten times less toxic than spiroxamine. Thus, the acute fish LC_{50} for M01, M02, M03 and M06 has been taken to be 2,410 µg/L and the acute aquatic invertebrate EC_{50} for M01, M02 and M06 has been taken to be 3000 µg/L.

Prothioconazole endpoints

For the toxicity endpoints of prothioconazole and the associated metabolites the endpoints have been taken directly from the 2007 EFSA Conclusion (EFSA Scientific Report (2007) 106, 1-98) without any further consideration. Risk assessments for prothioconazole have been presented here but only for completeness and to allow for the risk assessment of this representative formulation, containing spiroxamine, to be conducted. Discussion of the specific endpoints for prothioconazole are not considered part of the Renewal of Approval for spiroxamine. The EFSA Conclusion for prothioconazole also provides endpoints which have been conducted with a 250 EC formulation of prothioconazole which are not considered to be relevant for the risk assessment of Prothioconazole + Spiroxamine EC 460 and therefore these values have not been included in the tables above.

Formulation data

Studies have been conducted using the representative formulation, Prothioconazole + Spiroxamine EC 460, specifically an acute fish, acute *Daphnia*, algal and a *Lemna* study. These four studies provided a fish LC_{50} of 6,570 µg/L, a *Daphnia* EC_{50} of 6,300 µg/L, an algal E_rC_{50} of 147 µg/L and a *Lemna* E_rC_{50} of 54.2 µg/L. In order to determine whether or not the toxicity of the formulation reflects the combined effects of the two active substances, mixture toxicity calculations have been conducted and Model Deviation Ratios (MDR) determined, in accordance with the Aquatic Guidance Document. A separate assessment on combined mixture toxicity and a formulation specific risk assessment have been presented at the end of this section.

Exposure

FOCUS PEC_{SW} values have been determined for the proposed uses of Prothioconazole + Spiroxamine EC 460 on cereals at rates of 1 x 1.25 L/ha and 2 x 1.25 L/ha for both Spring and Winter cereals, considering early and late applications. Full details of the calculation of PEC_{SW} values, including FOCUS Step 4 values, have been presented in M-CP Section 9, Environmental fate.

PEC_{sw} values for prothioconazole and metabolites have been taken from the current draft RAR for spiroxamine (Spiroxamine dRAR, Volume 3, Annex B.9) and are considered to cover the proposed uses on cereals being assessed here. Please refer to Document M-CP Section 9 Environmental Fate for further details.

Isomers

In accordance with the isomer Guidance Document[5] it is necessary to consider the impact of selective degradation over time for isomeric substances, such as spiroxamine. In the absence of specific toxicity data on the individual isomers, an additional Uncertainty Factor (UF) is applied to the risk assessment if selective degradation could occur.

For parent spiroxamine further investigative environmental fate work is currently ongoing in order to clarify whether or not there is significant selective degradation of the individual spiroxamine isomers in surface water and sediment over time. Until this work has been completed and submitted a conclusion on the issue of selective degradation of spiroxamine in surface water cannot be made. Thus, for the risk assessment below an additional Uncertainty Factor (UF) has not been applied to the risk assessment for the parent materials spiroxamine and Spiroxamine EC 500 (*i.e.* an UF of 1.0 has been used).

For the metabolites of spiroxamine there are no chiral data available to be able to make an assessment over whether or not selective degradation occurs therefore there is a possibility that selective degradation of isomers could occur in surface water over time. As a conservative approach to account for any possible increased toxicity to aquatic organisms resulting from an increase in the ratio of a single isomer, an UF has been applied to the risk assessment of M01, M02, M03 and M06. The UF has been calculated following the recommendations of the isomer Guidance Document and have been presented in the table below.

Table CP 10.2-4 Uncertainty Factors determined for the aquatic toxicity data with the metabolites of spiroxamine

Test item	Study reference	Test material batch number	Isomer ratio	UF [1]
Acute fish				
M01	-	-	-	4.76 [2]
M02	-	-	-	12.5 [2]
M03	-	-	-	10.0 [2]
M06	-	-	-	2.32 [2]
Acute invertebrate				
M01	-	-	-	4.76 [2]
M02	-	-	-	12.5 [2]
M03	M-006702-01-1	950209ELB01	Not available	10.0 [3]
M06			-	2.32 [2]
Algae				
M01	M-288232-01-1	921103ELB02	A:B 56:42	4.76

[5] Guidance of EFSA on risk assessments for active substances of plant protection products that have stereoisomers as components or impurities and for transformation products of active substances that may have stereoisomers. EFSA Journal 2019;17(8):5804

Test item	Study reference	Test material batch number	Isomer ratio	UF [1]
M02	M-680695-01-2	AE 1344303-PU-01	A:B 83.1:16	12.5
M03	M-288235-01-1	KTS 10324-1-2	D1/D2/D3/D4: 27/26/20/27	10.0
M06	M-309818-01-1	SES 10277-2-1	A:B 43.04:52.75	2.32

[1] Changes in stereoisomeric excess are unknown therefore Uncertainty Factor = 100/ content of lowest stereoisomer (%) used for ecotox endpoint [as indicted in Table B.1, p.30 of isomer GD] and assumes that the toxicological effects of the mixture can be attributed to a single isomer. This assumes that all enantiomer ratios can be safely assumed to be 50:50. For example A:B ratio of 83.1:16 would be 100/(16/2) = UF of 12.5

[2] No toxicity data available for this metabolite for this organism group therefore the isomer ratio determined in the equivalent algal study has been used as a surrogate

[3] Toxicity data available with *Daphnia* for this metabolite but no isomer details available therefore the isomer ratio determined in the equivalent algal study has been used as a surrogate

- No toxicity data on metabolite available

Risk assessment

The risk assessment procedure follows the Aquatic Guidance Document (EFSA Journal 2013), as appropriate to the data requirements under EU Regulations 283/2013 and 284/2013.

The risk assessment has been presented using PEC/RAC ratios. For Spiroxamine, applications at 1 x 1.25 L/ha and 2 x 1.25 L/ha to Spring and Winter cereals have been considered in the risk assessment. Applications to early and to late growth stages have also been considered.

Risk assessments for spiroxamine and for prothioconazole have been presented separately using the available active substance and metabolite toxicity data. A separate formulation risk assessment has also been presented along with a consideration of predicted mixture toxicity.

Spiroxamine

1 x 1.25 L/ha - Spring cereals

Table CP 10.2-5 Aquatic organisms: acceptability of risk (PEC/RAC <1) for spiroxamine for each aquatic group based on FOCUS Steps 1 - 3 calculations for application of Prothioconazole + Spiroxamine EC 460 to Spring cereals (1 x 1.25 L/ha; early application)

Group		Fish acute	Fish chronic	Invertebrate acute	Invertebrate chronic
Test species		*Danio rerio*	*Danio rerio*	*Daphnia magna*	*Daphnia magna*
Endpoint (µg a.s./L)		LC_{50} 2410	EC_{10} 1.88	EC_{50} 3000	NOEC 34
AF		100	10	100	10
RAC (µg a.s./L)		24.1	0.188	30	3.4
FOCUS Scenario	PEC_{sw-max} (µg a.s./L)	PEC/RAC ratios			
Step 1	45.47	**1.89**	**242**	**1.52**	**13.4**
Step 2					
NEU	3.449	0.143	**18.3**	0.115	**1.01**

SEU	3.449	0.143	**18.3**	0.115	**1.01**
Step 3					
D1 Ditch	2.417	-	**12.9**	-	0.711
D1 Stream	2.096	-	**11.1**	-	0.616
D3 Ditch	2.369	-	**12.6**	-	0.697
D4 Pond	0.081	-	0.431	-	0.0238
D4 Stream	1.937	-	**10.3**	-	0.570
D5 Pond	0.082	-	0.436	-	0.0241
D5 Stream	1.989	-	**10.6**	-	0.585
R4 Stream	1.566	-	**8.33**	-	0.461

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the trigger of 1 are highlighted in **bold**

Table CP 10.2-5 (continued) Aquatic organisms: acceptability of risk (PEC/RAC <1) for spiroxamine for each aquatic group based on FOCUS Steps 1 - 3 calculations for application of Prothioconazole+

Spiroxamine EC 460
to Spring cereals (1 x 1.25 L/ha; early application)

Group		Algae (Freshwater)	Algae (Marine)	Mesocosm	Aquatic macrophytes	Sediment dweller	
Test species		***Scenedesmus subspicatus***	***Skeletonema costatum***	**Algae and invertebrates**	***Lemna gibba***	***Chironomus riparius***	***Lumbriculus variegatus***
Endpoint (µg a.s./L)		E_rC_{50} 12.0	E_rC_{50} 6.3	NOEC 1.0	EC_{50} 1910	NOEC 5600	EC_{10} 7120 µg/kg
AF		10	10	2	10	10	10
RAC (µg a.s./L)		1.2	0.63	0.5	191	560	712
FOCUS Scenario	PEC_{sw-max} (µg a.s./L)	PEC/RAC ratios					
Step 1	45.47	**37.9**	**72.2**	**90.9**	0.238	0.0812	**2.28**[1]
Step 2							
NEU	3.449	**2.87**	**5.47**	**6.90**	-	-	0.0926[2]
SEU	3.449	**2.87**	**5.47**	**6.90**	-	-	0.155[2]
Step 3							
D1 Ditch	2.417	**2.01**	**3.84**	**4.83**	-	-	-
D1 Stream	2.096	**1.75**	**3.33**	**4.19**	-	-	-
D3 Ditch	2.369	**1.97**	**3.76**	**4.74**	-	-	-
D4 Pond	0.081	0.0675	0.129	0.162	-	-	-
D4 Stream	1.937	**1.61**	**3.07**	**3.87**	-	-	-
D5 Pond	0.082	0.0683	0.130	0.164	-	-	-
D5 Stream	1.989	**1.66**	**3.16**	**3.98**	-	-	-
R4 Stream	1.566	**1.31**	**2.49**	**3.13**	-	-	-

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the trigger of 1 are highlighted in **bold**
[1] Based on a Step 1 PEC_{SED} of 1620 µg a.s./kg
[2] Based on Step 2 PEC_{SED} of 65.950 µg a.s./kg for NEU and 110.408 µg a.s./kg for SEU

Table CP 10.2.6A Aquatic organisms; acceptability of risk (PEC/RAC <1) for spiroxamine for each aquatic group based on FOCUS Steps 1 - 3 calculations for application of Prothioconazole + Spiroxamine EC 460 to Spring cereals (1 x 1.25 L/ha; late application)

Group	Fish acute	Fish chronic	Invertebrate acute	Invertebrate chronic
Test species	***Danio rerio***	***Danio rerio***	***Daphnia magna***	***Daphnia magna***
Endpoint (µg a.s./L)	LC_{50} 2410	EC_{10} 1.88	EC_{50} 3000	NOEC 34

AF		100	10	100	10
RAC (µg a.s./L)		24.1	0.188	30	3.4
FOCUS Scenario	PEC $_{sw-max}$ (µg a.s./L)	PEC/RAC ratios			
Step 1	45.47	**1.89**	**242**	**1.52**	**13.4**
Step 2					
NEU	3.449	0.143	**18.3**	0.115	**1.01**
SEU	3.449	0.143	**18.3**	0.115	**1.01**
Step 3					
D1 Ditch	2.414	-	**12.8**	-	0.710
D1 Stream	2.096	-	**11.1**	-	0.616
D3 Ditch	2.375	-	**12.6**	-	0.699
D4 Pond	0.081	-	0.431	-	0.0238
D4 Stream	2.048	-	**10.9**	-	0.602
D5 Pond	0.082	-	0.436	-	0.0241
D5 Stream	2.209	-	**11.8**	-	0.650
R4 Stream	1.810	-	**9.63**	-	0.532

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the trigger of 1 are highlighted in **bold**

Table CP 10.2-6 (continued) Aquatic organisms: acceptability of risk (PEC/RAC <1) for spiroxamine for each aquatic group based on FOCUS Steps 1 -3 calculations for application of Prothioconazole+ Spiroxamine EC 460 to Spring cereals (1 x 1.25 L/ha; late application)

Group		**Algae (Freshwater 1)**	**Algae (Marine)**	**Mesocosm**	**Aquatic macrophytes**	**Sediment dweller**	
Test species		***Scenedesmus subspicatus***	***Skeletonema costatum***	**Algae and invertebrates**	***Lemna gibba***	***Chironomus riparius***	***Lumbriculus variegatus***
Endpoint (µg a.s./L)		E_rC_{50} 12.0	E_rC_{50} 6.3	NOEC 1.0	EC_{50} 1910	NOEC 5600	EC_{10} 7120 µg/kg
AF		10	10	2	10	10	10
RAC (µg a.s./L)		1.2	0.63	0.5	191	560	712
FOCUS Scenario	PEC $_{sw-max}$ (µg a.s./L)	PEC/RAC ratios					
Step 1	45.47	**37.9**	**72.2**	**90.9**	0.238	0.0812	**2.28**[1]
Step 2							
NEU	3.449	**2.87**	**5.47**	**6.90**	-	-	0.0926[2]

SEU	3.449	**2.87**	**5.47**	**6.90**	-	-	0.155[2]
Step 3							
D1 Ditch	2.414	**2.01**	**3.83**	**4.83**	-	-	-
D1 Stream	2.096	**1.75**	**3.33**	**4.19**	-	-	-
D3 Ditch	2.375	**1.98**	**3.77**	**4.75**	-	-	-
D4 Pond	0.081	0.0675	0.129	0.162	-	-	-
D4 Stream	2.048	**1.71**	**3.25**	**4.10**	-	-	-
D5 Pond	0.082	0.0683	0.130	0.164	-	-	-
D5 Stream	2.209	**1.84**	**3.51**	**4.42**	-	-	-
R4 Stream	1.810	**1.51**	**2.87**	**3.62**	-	-	-

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the trigger of 1 are highlighted in **bold**
[1] Based on a Step 1 PEC_{SED} of 1620 µg a.s./kg
[2] Based on Step 2 PEC_{SED} of 65.950 µg a.s./kg for NEU and 110.408 µg a.s./kg for SEU

For the acute fish, acute invertebrate, aquatic macrophyte and sediment-dweller risk assessments, the PEC/RAC ratios are <1 using FOCUS Step 1 or Step 2 PEC_{sw} values thereby demonstrating an acceptable risk to these organism groups from exposure to spiroxamine following application of Prothioconazole + Spiroxamine EC 460 to Spring cereals at 1 x 1.25 L/ha (early and late applications).

For the chronic invertebrate risk assessment an acceptable risk could be demonstrated using FOCUS Step 3 PEC_{sw} values for early and late applications to Spring cereals at 1 x 1.25 L/ha.

For the chronic fish and algal risk assessments, as well as those organisms covered by the mesocosm study, some FOCUS scenarios passed the risk assessment when Step 3 PEC_{sw} values were used but the majority of FOCUS scenarios for these groups require refinement of the risk assessment. Refined risk assessments using FOCUS Step 4 PEC_{sw} values are presented later in this section.

1 x 1.25 L/ha – Winter cereals

Table CP 10.2-7 Aquatic organisms: acceptability of risk (PEC/RAC <1) for spiroxamine for each aquatic group based on FOCUS Steps 1 - 3 calculations for application of Prothioconazole + Spiroxamine EC 460 to Winter cereals (1 x 1.25 L/ha; early application)

Group		**Fish acute**	**Fish chronic**	**Invertebrate acute**	**Invertebrate chronic**
Test species		***Danio rerio***	***Danio rerio***	***Daphnia magna***	***Daphnia magna***
Endpoint (µg a.s./L)		LC_{50} 2410	EC_{10} 1.88	EC_{50} 3000	NOEC 34
AF		100	10	100	10
RAC (µg a.s./L)		24.1	0.188	30	3.4
FOCUS Scenario	PEC_{sw-max} (µg a.s./L)	PEC/RAC ratios			
Step 1	45.47	**1.89**	**242**	**1.52**	**13.4**
Step 2					

NEU	3.449	0.143	**18.3**	0.115	**1.01**
SEU	3.449	0.143	**18.3**	0.115	**1.01**
Step 3					
D1 Ditch	2.370	-	**12.6**	-	0.697
D1 Stream	1.843	-	**9.80**	-	0.542
D2 Ditch	2.392	-	**12.7**	-	0.704
D2 Stream	2.113	-	**11.2**	-	0.621
D3 Ditch	2.361	-	**12.6**	-	0.694
D4 Pond	0.081	-	0.431	-	0.0238
D4 Stream	1.745	-	**9.28**	-	0.513
D5 Pond	0.081	-	0.431	-	0.0238
D5 Stream	1.885	-	**10.0**	-	0.554
D6 Ditch	2.334	-	**12.4**	-	0.686
R1 Pond	0.081	-	0.431	-	0.0238
R1 Stream	1.555	-	**8.27**	-	0.457
R3 Stream	2.185	-	**11.6**	-	0.643
R4 Stream	1.562	-	**8.31**	-	0.459

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the trigger of 1 are highlighted in **bold**

Table CP 10.2-7 (continued) Aquatic organisms: acceptability of risk (PEC/RAC <1) for spiroxamine for each aquatic group based on FOCUS Steps 1 - 3 calculations for application of Prothioconazole+ Spiroxamine EC 460 to Winter cereals (1 x 1.25 L/ha; early application)

Group		**Algae (Freshwater)**	**Algae (Marine)**	**Mesocosm**	**Aquatic macrophytes**	**Sediment dweller**	
Test species		***Scenedesmus subspicatus***	***Skeletonema costatum***	**Algae and invertebrates**	***Lemna gibba***	***Chironomus riparius***	***Lumbriculus variegatus***
Endpoint (µg a.s./L)		E_rC_{50} 12.0	E_rC_{50} 6.3	NOEC 1.0	EC_{50} 1910	NOEC 5600	EC_{10} 7120 µg/kg
AF		10	10	2	10	10	10
RAC (µg a.s./L)		1.2	0.63	0.5	191	560	712
FOCUS Scenario	PEC_{sw-max} (µg a.s./L)	PEC/RAC ratios					
Step 1	45.47	**37.9**	**72.2**	**90.9**	0.238	0.0812	**2.28**[1]
Step 2							
NEU	3.449	**2.87**	**5.47**	**6.90**	-	-	0.0926[2]

SEU	3.449	**2.87**	**5.47**	**6.90**	-	-	0.155[2]
Step 3							
D1 Ditch	2.370	**1.98**	**3.76**	**4.74**	-	-	-
D1 Stream	1.843	**1.54**	**2.93**	**3.69**	-	-	-
D2 Ditch	2.392	**1.99**	**3.80**	**4.78**	-	-	-
D2 Stream	2.113	**1.76**	**3.35**	**4.23**	-	-	-
D3 Ditch	2.361	**1.97**	**3.75**	**4.72**	-	-	-
D4 Pond	0.081	0.0675	0.129	0.162	-	-	-
D4 Stream	1.745	**1.45**	**2.77**	**3.49**	-	-	-
D5 Pond	0.081	0.0675	0.129	0.162	-	-	-
D5 Stream	1.885	**1.57**	**2.99**	**3.77**	-	-	-
D6 Ditch	2.334	**1.95**	**3.70**	**4.67**	-	-	-
R1 Pond	0.081	0.0675	0.129	0.162	-	-	-
R1 Stream	1.555	**1.30**	**2.47**	**3.11**	-	-	-
R3 Stream	2.185	**1.82**	**3.47**	**4.37**	-	-	-
R4 Stream	1.562	**1.30**	**2.48**	**3.12**	-	-	-

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the trigger of 1 are highlighted in **bold**

[1] Based on a Step 1 PEC_{SED} of 1620 µg a.s./kg

[2] Based on Step 2 PEC_{SED} of 65.950 µg a.s./kg for NEU and 110.408 µg a.s./kg for SEU

Table CP 10.2-8 Aquatic organisms: acceptability of risk (PEC/RAC <1) for spiroxamine for each aquatic group based on FOCUS Steps 1 - 3 calculations for application of Prothioconazole + Spiroxamine EC 460 to Winter cereals (1 x 1.25 L/ha; late application)

Group		**Fish acute**	**Fish chronic**	**Invertebrate acute**	**Invertebrate chronic**
Test species		***Danio rerio***	***Danio rerio***	***Daphnia magna***	***Daphnia magna***
Endpoint (µg a.s./L)		LC_{50} 2410	EC_{10} 188	EC_{50} 3000	NOEC 34
AF		100	10	100	10
RAC (µg a.s./L)		24.1	0.188	30	3.4
FOCUS Scenario	$PEC_{sw\ max}$ (µg a.s./L)	PEC/RAC ratios			
Step 1	45.47	**1.89**	**242**	**1.52**	**13.4**
Step 2					
NEU	3.449	0.143	**18.3**	0.115	**1.01**
SEU	3.449	0.143	**18.3**	0.115	**1.01**
Step 3					

D1 Ditch	2.391	-	**12.7**	-	0.703
D1 Stream	2.091	-	**11.1**	-	0.615
D2 Ditch	2.393	-	**12.7**	-	0.704
D2 Stream	2.129	-	**11.3**	-	0.626
D3 Ditch	2.372	-	**12.6**	-	0.698
D4 Pond	0.081	-	0.431	-	0.0238
D4 Stream	2.043	-	**10.9**	-	0.601
D5 Pond	0.081	-	0.431	-	0.0238
D5 Stream	2.204	-	**11.7**	-	0.648
D6 Ditch	2.383	-	**12.7**	-	0.701
R1 Pond	0.169	-	0.899	-	0.0497
R1 Stream	1.562	-	**8.31**	-	0.459
R3 Stream	2.203	-	**11.7**	-	0.648
R4 Stream	1.562	-	**8.31**	-	0.459

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the trigger of 1 are highlighted in **bold**

Table CP 10.2-8 (continued) Aquatic organisms: acceptability of risk (PEC/RAC <1) for spiroxamine for each aquatic group based on FOCUS Steps 1 - 3 calculations for application of Prothioconazole + Spiroxamine EC 460 to Winter cereals (1 x 1.25 L/ha; late application)

Group		**Algae (Freshwater)**	**Algae (Marine)**	**Mesocosm**	**Aquatic macrophytes**	**Sediment dweller**	
Test species		***Scenedesmus subspicatus***	***Skeletonema costatum***	**Algae and invertebrates**	***Lemna gibba***	***Chironomus riparius***	***Lumbriculus variegatus***
Endpoint (µg a.s./L)		E_rC_{50} 12.0	E_rC_{50} 6.3	NOEC 1.0	EC_{50} 1910	NOEC 5600	EC_{10} 7120 µg/kg
AF		10	10	2	10	10	10
RAC (µg a.s./L)		1.2	0.63	0.5	191	560	712
FOCUS Scenario	PEC_{sw-max} (µg a.s./L)	PEC/RAC ratios					
Step 1	45.47	**37.9**	**72.2**	**90.9**	0.238	0.0812	**2.28**[1]
Step 2							
NEU	3.449	**2.87**	**5.47**	**6.90**	-	-	0.0926[2]
SEU	3.449	**2.87**	**5.47**	**6.90**	-	-	0.155[2]
Step 3							
D1 Ditch	2.391	**1.99**	**3.80**	**4.78**	-	-	-

D1 Stream	2.091	**1.74**	**3.32**	**4.18**	-	-	-
D2 Ditch	2.393	**1.99**	**3.80**	**4.79**	-	-	-
D2 Stream	2.129	**1.77**	**3.38**	**4.26**	-	-	-
D3 Ditch	2.372	**1.98**	**3.77**	**4.74**	-	-	-
D4 Pond	0.081	0.0675	0.129	0.162	-	-	-
D4 Stream	2.043	**1.70**	**3.24**	**4.09**	-	-	-
D5 Pond	0.081	0.0675	0.129	0.162	-	-	-
D5 Stream	2.204	**1.84**	**3.50**	**4.41**	-	-	-
D6 Ditch	2.383	**1.99**	**3.78**	**4.77**	-	-	-
R1 Pond	0.169	0.141	0.268	0.338	-	-	-
R1 Stream	1.562	**1.30**	**2.48**	**3.12**	-	-	-
R3 Stream	2.203	**1.84**	**3.50**	**4.41**	-	-	-
R4 Stream	1.562	**1.30**	**2.48**	**3.12**	-	-	-

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the trigger of 1 are highlighted in **bold**
[1] Based on a Step 1 PEC_{SED} of 1620 µg a.s./kg
[2] Based on Step 2 PEC_{SED} of 65.950 µg a.s./kg for NEU and 110.408 µg a.s./kg for SEU

For the acute fish, acute invertebrate, aquatic macrophyte and sediment-dweller risk assessments, the PEC/RAC ratios are <1 using FOCUS Step 1 or Step 2 PEC_{sw} values, thereby demonstrating an acceptable risk to these organism groups from exposure to spiroxamine following application of Prothioconazole + Spiroxamine EC 460 to Winter cereals at 1 x 1.25 L/ha (early and late applications).

For the chronic invertebrate risk assessment an acceptable risk could be demonstrated using FOCUS Step 3 PEC_{sw} values for early and late applications to Winter cereals at 1 x 1.25 L/ha.

For the chronic fish and algal risk assessments, as well as those organisms covered by the mesocosm study, some FOCUS scenarios passed the risk assessment when Step 3 PEC_{sw} values were used but the majority of FOCUS scenarios for these groups require refinement of the risk assessment. Refined risk assessments using FOCUS Step 4 PEC_{sw} values are presented later in this section.

2 x 1.25 L/ha – Spring cereals

Table CP 10.2-9 Aquatic organisms: acceptability of risk (PEC/RAC <1) for spiroxamine for each aquatic group based on FOCUS Steps 1–3 calculations for application of Prothioconazole + Spiroxamine EC 460 to Spring cereals (2 x 1.25 L/ha; early application)

Group		**Fish acute**	**Fish chronic**	**Invertebrate acute**	**Invertebrate chronic**
Test species		***Danio rerio***	***Danio rerio***	***Daphnia magna***	***Daphnia magna***
Endpoint (µg a.s./L)		LC_{50} 2410	EC_{10} 1.88	EC_{50} 3000	NOEC 34
AF		100	10	100	10
RAC (µg a.s./L)		24.1	0.188	30	3.4
FOCUS Scenario	PEC_{sw-max} (µg a.s./L)	PEC/RAC ratios			

Step 1	45.47	**1.89**	**242**	**1.52**	**13.4**
Step 2					
NEU	3.673	0.152	**19.5**	0.122	**1.08**
SEU	5.194	0.216	**27.6**	0.173	**1.53**
Step 3					
D1 Ditch	3.139	-	**16.7**	-	0.923
D1 Stream	2.096	-	**11.1**	-	0.616
D3 Ditch	2.369	-	**12.6**	-	0.697
D4 Pond	0.111	-	0.590	-	0.0326
D4 Stream	1.937	-	**10.3**	-	0.570
D5 Pond	0.106	-	0.564	-	0.0312
D5 Stream	1.989	-	**10.6**	-	0.585
R4 Stream	3.063	-	**16.3**	-	0.901

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the trigger of 1 are highlighted in **bold**

Table CP 10.2-9 (continued) Aquatic organisms: acceptability of risk (PEC/RAC <1) for spiroxamine for each aquatic group based on FOCUS Steps 1 - 3 calculations for application of Prothioconazole+ Spiroxamine EC 460 to Spring cereals (2 x 1.25 L/ha; early application)

Group		**Algae (Freshwater)**	**Algae (Marine)**	**Mesocosm**	**Aquatic macrophytes**	**Sediment dweller**	
Test species		***Scenedesmus subspicatus***	***Skeletonema costatum***	**Algae and invertebrates**	***Lemna gibba***	***Chironomus riparius***	***Lumbriculus variegatus***
Endpoint (µg a.s./L)		E_rC_{50} 12.0	E_rC_{50} 6.3	NOEC 1.0	EC_{50} 1910	NOEC 5600	EC_{10} 7120 µg/kg
AF		10	10	2	10	10	10
RAC (µg a.s./L)		1.2	0.63	0.5	191	560	712
FOCUS Scenario	PEC_{sw-max} (µg a.s./L)	PEC/RAC ratios					
Step 1	45.47	**37.9**	**72.2**	**90.9**	0.238	0.0812	**2.28**[1]
Step 2							
NEU	3.673	**3.06**	**5.83**	**7.35**	-	-	0.165[2]
SEU	5.194	**4.33**	**8.24**	**10.4**	-	-	0.277[2]
Step 3							
D1 Ditch	3.139	**2.62**	**4.98**	**6.28**	-	-	-
D1 Stream	2.096	**1.75**	**3.33**	**4.19**	-	-	-

D3 Ditch	2.369	**1.97**	**3.76**	**4.74**	-	-	-
D4 Pond	0.111	0.0925	0.176	0.222	-	-	-
D4 Stream	1.937	**1.61**	**3.07**	**3.87**	-	-	-
D5 Pond	0.106	0.0883	0.168	0.212	-	-	-
D5 Stream	1.989	**1.66**	**3.16**	**3.98**	-	-	-
R4 Stream	3.063	**2.55**	**4.86**	**6.13**	-	-	-

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the trigger of 1 are highlighted in **bold**
[1] Based on a Step 1 PEC_{SED} of 1620 µg a.s./kg
[2] Based on Step 2 PEC_{SED} of 117.133 µg a.s./kg for NEU and 197.213 µg a.s./kg for SEU

Table CP 10.2-10 Aquatic organisms: acceptability of risk (PEC/RAC <1) for spiroxamine for each aquatic group based on FOCUS Steps 1 - 3 calculations for application of Prothioconazole + Spiroxamine EC 460 to Spring cereals (2 x 1.25 L/ha; late application)

Group		**Fish acute**	**Fish chronic**	**Invertebrate acute**	**Invertebrate chronic**
Test species		***Danio rerio***	***Danio rerio***	***Daphnia magna***	***Daphnia magna***
Endpoint (µg a.s./L)		LC_{50} 2410	EC_{10} 1.88	EC_{50} 3000	NOEC 34
AF		100	10	100	10
RAC (µg a.s./L)		24.1	0.188	30	3.4
FOCUS Scenario	PEC_{sw-max} (µg a.s./L)	PEC/RAC ratios			
Step 1	45.47	**1.89**	**242**	**1.52**	**13.4**
Step 2					
NEU	3.673	0.152	**19.5**	0.122	**1.08**
SEU	5.194	0.216	**27.6**	0.173	**1.53**
Step 3					
D1 Ditch	2.834		**15.1**		0.834
D1 Stream	2.096		**11.1**		0.616
D3 Ditch	2.375		**12.6**		0.699
D4 Pond	0.114		0.606		0.0335
D4 Stream	2.048		**10.9**		0.602
D5 Pond	0.114		0.606		0.0335
D5 Stream	2.209		**11.8**		0.650
R4 Stream	1.961		**10.4**		0.577

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the trigger of 1 are highlighted in **bold**

Table CP 10.2-10 (continued) Aquatic organisms: acceptability of risk (PEC/RAC <1) for spiroxamine for each aquatic group based on FOCUS Steps 1 - 3 calculations for application of Prothioconazole+ Spiroxamine EC 460 to Spring cereals (2 x 1.25 L/ha; late application)

Group		**Algae (Freshwater)**	**Algae (Marine)**	**Mesocosm**	**Aquatic macrophytes**	**Sediment dweller**	
Test species		***Scenedesmus subspicatus***	***Skeletonema costatum***	**Algae and invertebrates**	***Lemna gibba***	***Chironomus riparius***	***Lumbriculus variegatus***
Endpoint (µg a.s./L)		E_rC_{50} 12.0	E_rC_{50} 6.3	NOEC 10	EC_{50} 1910	NOEC 5600	EC_{10} 7120 µg/kg
AF		10	10	2	10	10	10
RAC (µg a.s./L)		1.2	0.63	5	191	560	712
FOCUS Scenario	PEC_{sw-max} (µg a.s./L)	PEC/RAC ratios					
Step 1	45.47	**37.9**	**72.2**	**90.9**	0.238	0.0812	**2.28**[1]
Step 2							
NEU	3.673	**3.06**	**5.83**	**7.35**	-	-	0.165[2]
SEU	5.194	**4.33**	**8.24**	**10.4**	-	-	0.277[2]
Step 3							
D1 Ditch	2.834	**2.36**	**4.50**	**5.67**	-	-	-
D1 Stream	2.096	**1.75**	**3.33**	**4.19**	-	-	-
D3 Ditch	2.375	**1.98**	**3.77**	**4.75**	-	-	-
D4 Pond	0.114	0.0950	0.181	0.228	-	-	-
D4 Stream	2.048	**1.71**	**3.25**	**4.10**	-	-	-
D5 Pond	0.114	0.0950	0.181	0.228	-	-	-
D5 Stream	2.209	**1.84**	**3.51**	**4.42**	-	-	-
R4 Stream	1.961	**1.63**	**3.11**	**3.92**	-	-	-

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the trigger of 1 are highlighted in **bold**

[1] Based on a Step 1 PEC_{SED} of 1620 µg a.s./kg

[2] Based on Step 2 PEC_{SED} of 117.133 µg a.s./kg for NEU and 197.213 µg a.s./kg for SEU

For the acute fish, acute invertebrate, aquatic macrophyte and sediment-dweller risk assessments, the PEC/RAC ratios are <1 using FOCUS Step 1 or Step 2 PEC_{sw} values thereby demonstrating an acceptable risk to these organism groups from exposure to spiroxamine following application of Prothioconazole + Spiroxamine EC 460 to Spring cereals at 2 x 1.25 L/ha (early and late applications).

For the chronic invertebrate risk assessment an acceptable risk could be demonstrated using FOCUS Step 3 PEC_{sw} values for early and late applications to Spring cereals at 2 x 1.25 L/ha.

For the chronic fish and algal risk assessments, as well as those organisms covered by the mesocosm study, some FOCUS scenarios passed the risk assessment when Step 3 PEC_{sw} values were used but the majority of FOCUS scenarios for these groups require refinement of the risk assessment. Refined risk assessments using FOCUS Step 4 PEC_{sw} values are presented later in this section.

2 x 1.25 L/ha – Winter cereals

Table CP 10.2-11 Aquatic organisms: acceptability of risk (PEC/RAC <1) for spiroxamine for each aquatic group based on FOCUS Steps 1 - 3 calculations for application of Prothioconazole + Spiroxamine EC 460 to Winter cereals (2 x 1.25 L/ha; early application)

Group		**Fish acute**	**Fish chronic**	**Invertebrate acute**	**Invertebrate chronic**
Test species		***Danio rerio***	***Danio rerio***	***Daphnia magna***	***Daphnia magna***
Endpoint (µg a.s./L)		LC_{50} 2410	EC_{10} 1.88	EC_{50} 3000	NOEC 34
AF		100	10	100	10
RAC (µg a.s./L)		24.1	0.188	30	3.4
FOCUS Scenario	PEC_{sw-max} (µg a.s./L)	PEC/RAC ratios			
Step 1	45.47	**1.89**	**242**	**1.52**	**13.4**
Step 2					
NEU	3.673	0.152	**19.5**	0.122	**1.08**
SEU	5.194	0.216	**27.6**	0.173	**1.53**
Step 3					
D1 Ditch	2.370	-	**12.6**	-	0.697
D1 Stream	1.843	-	**9.80**	-	0.542
D2 Ditch	2.392	-	**12.7**	-	0.704
D2 Stream	2.113	-	**11.2**	-	0.621
D3 Ditch	2.361	-	**12.6**	-	0.694
D4 Pond	0.100	-	0.532	-	0.0294
D4 Stream	1.745	-	**9.28**	-	0.513
D5 Pond	0.113	-	0.601	-	0.0332
D5 Stream	1.885	-	**10.0**	-	0.554
D6 Ditch	2.334	-	**12.4**	-	0.686
R1 Pond	0.184	-	0.979	-	0.0541
R1 Stream	1.555	-	**8.27**	-	0.457
R3 Stream	2.185	-	**11.6**	-	0.643
R4 Stream	1.666	-	**8.86**	-	0.490

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the trigger of 1 are highlighted in **bold**

Table CP 10.2-11 (continued) Aquatic organisms: acceptability of risk (PEC/RAC <1) for spiroxamine for each aquatic group based on FOCUS Steps 1 - 3 calculations for application of Prothioconazole+ Spiroxamine EC 460 to Winter cereals (2 x 1.25 L/ha; early application)

Group		**Algae (Freshwater)**	**Algae (Marine)**	**Mesocosm**	**Aquatic macrophytes**	**Sediment dweller**	
Test species		***Scenedesmus subspicatus***	***Skeletonema costatum***	**Algae and invertebrates**	***Lemna gibba***	***Chironomus riparius***	***Lumbriculus variegatus***
Endpoint (µg a.s./L)		E_rC_{50} 12.0	E_rC_{50} 6.3	NOEC 10	EC_{50} 1910	NOEC 5600	EC_{10} 7120 µg/kg
AF		10	10	2	10	10	10
RAC (µg a.s./L)		1.2	0.63	5	191	560	712
FOCUS Scenario	PEC_{sw-max} (µg a.s./L)	PEC/RAC ratios					
Step 1	45.47	**37.9**	**72.2**	**90.9**	0.238	0.0812	**2.28**[1]
Step 2							
NEU	3.673	**3.06**	**5.83**	**7.35**	-	-	0.165[2]
SEU	5.194	**4.33**	**8.24**	**10.4**	-	-	0.277[2]
Step 3							
D1 Ditch	2.370	**1.98**	**3.76**	**4.74**	-	-	-
D1 Stream	1.843	**1.54**	**2.93**	**3.69**	-	-	-
D2 Ditch	2.392	**1.99**	**3.80**	**4.78**	-	-	-
D2 Stream	2.113	**1.76**	**3.35**	**4.23**	-	-	-
D3 Ditch	2.361	**1.97**	**3.75**	**4.72**	-	-	-
D4 Pond	0.100	0.0833	0.159	0.200	-	-	-
D4 Stream	1.745	**1.45**	**2.77**	**3.49**	-	-	-
D5 Pond	0.113	0.0942	0.179	0.226	-	-	-
D5 Stream	1.885	**1.57**	**2.99**	**3.77**	-	-	-
D6 Ditch	2.334	**1.95**	**3.70**	**4.67**	-	-	-
R1 Pond	0.184	0.153	0.292	0.368	-	-	-
R1 Stream	1.555	**1.30**	**2.47**	**3.11**	-	-	-
R3 Stream	2.185	**1.82**	**3.47**	**4.37**	-	-	-
R4 Stream	1.666	**1.39**	**2.64**	**3.33**	-	-	-

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the trigger of 1 are highlighted in **bold**

[1] Based on a Step 1 PEC_{SED} of 1620 µg a.s./kg

[2] Based on Step 2 PEC_{SED} of 117.133 µg a.s./kg for NEU and 197.213 µg a.s./kg for SEU

Table CP 10.2-12 Aquatic organisms: acceptability of risk (PEC/RAC <1) for spiroxamine for each aquatic group based on FOCUS Steps 1 - 3 calculations for application of Prothioconazole + Spiroxamine EC 460 to Winter cereals (2 x 1.25 L/ha; late application)

Group		**Fish acute**	**Fish chronic**	**Invertebrate acute**	**Invertebrate chronic**
Test species		***Danio rerio***	***Danio rerio***	***Daphnia magna***	***Daphnia magna***
Endpoint (µg a.s./L)		LC_{50} 2410	EC_{10} 1.88	EC_{50} 3000	NOEC 34
AF		100	10	100	10
RAC (µg a.s./L)		24.1	0.188	30	3.4
FOCUS Scenario	PEC_{sw-max} (µg a.s./L)	PEC/RAC ratios			
Step 1	45.47	**1.89**	**242**	**1.52**	**13.4**
Step 2					
NEU	3.673	0.152	**19.5**	0.122	**1.08**
SEU	5.194	0.216	**27.6**	0.173	**1.53**
Step 3					
D1 Ditch	2.967	-	**15.8**	-	0.873
D1 Stream	2.091	-	**11.1**	-	0.615
D2 Ditch	2.994	-	**15.9**	-	0.881
D2 Stream	2.619	-	**13.9**	-	0.770
D3 Ditch	2.372	-	**12.6**	-	0.698
D4 Pond	0.113	-	0.601	-	0.0332
D4 Stream	2.043	-	**10.9**	-	0.601
D5 Pond	0.112	-	0.596	-	0.0329
D5 Stream	2.204	-	**11.7**	-	0.648
D6 Ditch	2.383	-	**12.7**	-	0.701
R1 Pond	0.296	-	**1.57**	-	0.0871
R1 Stream	1.562	-	**8.31**	-	0.459
R3 Stream	2.203	-	**11.7**	-	0.648
R4 Stream	1.562	-	**8.31**	-	0.459

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the trigger of 1 are highlighted in **bold**

Table CP 10.2-12 (continued) Aquatic organisms: acceptability of risk (PEC/RAC <1) for spiroxamine for each aquatic group based on FOCUS Steps 1 - 3 calculations for application of Prothioconazole+ Spiroxamine EC 460 to Winter cereals (2 x 1.25 L/ha; late application)

Group		**Algae (Freshwater)**	**Algae (Marine)**	**Mesocosm**	**Aquatic macrophytes**	**Sediment dweller**	
Test species		***Scenedesmus subspicatus***	***Skeletonema costatum***	**Algae and invertebrates**	***Lemna gibba***	***Chironomus riparius***	***Lumbriculus variegatus***
Endpoint (µg a.s./L)		E_rC_{50} 12.0	E_rC_{50} 6.3	NOEC 10	EC_{50} 1910	NOEC 5600	EC_{10} 7120 µg/kg
AF		10	10	2	10	10	10
RAC (µg a.s./L)		1.2	0.63	5	191	560	712
FOCUS Scenario	PEC_{sw-max} (µg a.s./L)	PEC/RAC ratios					
Step 1	45.47	**37.9**	**72.2**	**90.9**	0.238	0.0812	**2.28**[1]
Step 2							
NEU	3.673	**3.06**	**5.83**	**7.35**	-	-	0.165[2]
SEU	5.194	**4.33**	**8.24**	**10.4**	-	-	0.277[2]
Step 3							
D1 Ditch	2.967	**2.47**	**4.71**	**5.93**	-	-	-
D1 Stream	2.091	**1.74**	**3.32**	**4.18**	-	-	-
D2 Ditch	2.994	**2.50**	**4.75**	**5.99**	-	-	-
D2 Stream	2.619	**2.18**	**4.16**	**5.24**	-	-	-
D3 Ditch	2.372	**1.98**	**3.77**	**4.74**	-	-	-
D4 Pond	0.113	0.0942	0.179	0.226	-	-	-
D4 Stream	2.043	**1.70**	**3.24**	**4.09**	-	-	-
D5 Pond	0.112	0.0933	0.178	0.224	-	-	-
D5 Stream	2.204	**1.84**	**3.50**	**4.41**	-	-	-
D6 Ditch	2.383	**1.99**	**3.78**	**4.77**	-	-	-
R1 Pond	0.296	0.247	0.470	0.592	-	-	-
R1 Stream	1.562	**1.30**	**2.48**	**3.12**	-	-	-
R3 Stream	2.203	**1.84**	**3.50**	**4.41**	-	-	-
R4 Stream	1.562	**1.30**	**2.48**	**3.12**	-	-	-

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the trigger of 1 are highlighted in **bold**

[1] Based on a Step 1 PEC_{SED} of 1620 µg a.s./kg

[2] Based on Step 2 PEC_{SED} of 117.133 µg a.s./kg for NEU and 197.213 µg a.s./kg for SEU

For the acute fish, acute invertebrate, aquatic macrophyte and sediment-dweller risk assessments, the PEC/RAC ratios are <1 using FOCUS Step 1 or Step 2 PEC_{sw} values thereby demonstrating an

acceptable risk to these organism groups from exposure to spiroxamine following application of Prothioconazole + Spiroxamine EC 460 to Winter cereals at 2 x 1.25 L/ha (early and late applications).

For the chronic invertebrate risk assessment an acceptable risk could be demonstrated using FOCUS Step 3 PEC_{sw} values for early and late applications to Winter cereals at 2 x 1.25 L/ha.

For the chronic fish and algal risk assessments, as well as those organisms covered by the mesocosm study, some FOCUS scenarios passed the risk assessment when Step 3 PEC_{sw} values were used but the majority of FOCUS scenarios for these groups require refinement of the risk assessment. Refined risk assessments using FOCUS Step 4 PEC_{sw} values are presented below.

Refined risk assessment for spiroxamine using Step 4 PEC_{sw} values

For each of the proposed uses of Prothioconazole + Spiroxamine EC 460, refined risk assessments for those organism groups that did not pass the risk assessment using Step 3 PEC_{sw} values for all of the relevant FOCUS scenarios have been presented below. The exposure estimates have been refined by use of Step 4 PEC_{sw} values considering mitigation measures in the form of either *i)*: a 20 m no-spray buffer zone with a 20 m vegetated filter strip or *ii)*: a 30 m no-spray buffer zone with a 20 m vegetated filter strip.

Table CP 10.2-13 Aquatic organisms: acceptability of risk (PEC/RAC <1) for spiroxamine based on FOCUS Step 4 calculations for application of Prothioconazole + Spiroxamine EC 460 to Spring cereals (1 x 1.25 L/ha; early application)

Group		Fish chronic	Algae (Freshwater)	Algae (Marine)	Mesocosm
Test species		***Danio rerio***	***Scenedesmus subspicatus***	***Skeletonema costatum***	**Algae and invertebrates**
Endpoint (µg a.s./L)		EC_{10} 1.88	E_rC_{50} 12.0	E_rC_{50} 6.3	NOEC 1.0
AF		10	10	10	2
RAC (µg a.s./L)		0.188	1.2	0.63	0.5
FOCUS Scenario	PEC_{sw-max} (µg a.s./L)	PEC/RAC ratios			
Step 4 (20 m nsbz and 20 m vfs)					
D1 Ditch	0.244	**1.30**	0.203	0.387	0.488
D1 Stream	0.211	**1.12**	0.176	0.335	0.422
D3 Ditch	0.182	0.968	0.152	0.289	0.364
D4 Pond	0.046	-	-	-	-
D4 Stream	0.200	**1.06**	0.167	0.317	0.400
D5 Pond	0.046	-	-	-	-
D5 Stream	0.205	**1.09**	0.171	0.325	0.410
R4 Stream	0.173	0.920	0.144	0.275	0.346
Step 4 (30 m nsbz and 20 m vfs)					
D1 Ditch	0.192	**1.02**	-	-	-
D1 Stream	0.143	0.761	-	-	-
D3 Ditch	0.138	-	-	-	-
D4 Pond	0.038	-	-	-	-

Group		Fish chronic	Algae (Freshwater)	Algae (Marine)	Mesocosm
Test species		*Danio rerio*	*Scenedesmus subspicatus*	*Skeletonema costatum*	Algae and invertebrates
D4 Stream	0.138	0.734	-	-	-
D5 Pond	0.038	-	-	-	-
D5 Stream	0.140	0.745	-	-	-
R4 Stream	0.173	-	-	-	-

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the trigger of 1 are highlighted in **bold**; nsbz: no spray buffer zone; vfs: vegetated filter strip; - not required as risk assessment already passes

Table CP 10.2-14 Aquatic organisms: acceptability of risk (PEC/RAC <1) for spiroxamine based on FOCUS Step 4 calculations for application of Prothioconazole + Spiroxamine EC 460 to Spring cereals (1 x 1.25 L/ha; late application)

Group		Fish chronic	Algae (Freshwater)	Algae (Marine)	Mesocosm
Test species		*Danio rerio*	*Scenedesmus subspicatus*	*Skeletonema costatum*	Algae and invertebrates
Endpoint (µg a.s./L)		EC_{10} 1.88	E_rC_{50} 12.0	E_rC_{50} 6.3	NOEC 1.0
AF		10	10	10	2
RAC (µg a.s./L)		0.188	1.2	0.63	0.5
FOCUS Scenario	PEC_{sw-max} (µg a.s./L)	PEC/RAC ratios			
Step 4 (20 m nsbz and 20 m vfs)					
D1 Ditch	0.244	**1.30**	0.203	0.387	0.488
D1 Stream	0.211	**1.12**	0.176	0.335	0.422
D3 Ditch	0.194	**1.03**	0.162	0.308	0.388
D4 Pond	0.046	-	-	-	-
D4 Stream	0.210	**1.12**	0.175	0.333	0.420
D5 Pond	0.046	-	-	-	-
D5 Stream	0.224	**1.19**	0.187	0.356	0.448
R4 Stream	0.224	**1.19**	0.187	0.356	0.448
Step 4 (30 m nsbz and 20 m vfs)					
D1 Ditch	0.192	**1.02**	-	-	-
D1 Stream	0.143	0.761	-	-	-
D3 Ditch	0.154	0.819	-	-	-
D4 Pond	0.038	-	-	-	-
D4 Stream	0.145	0.771	-	-	-

Group		Fish chronic	Algae (Freshwater)	Algae (Marine)	Mesocosm
Test species		*Danio rerio*	*Scenedesmus subspicatus*	*Skeletonema costatum*	Algae and invertebrates
D5 Pond	0.038	-	-	-	-
D5 Stream	0.155	0.824	-	-	-
R4 Stream	0.224	**1.19**	-	-	-

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the trigger of 1 are highlighted in **bold**; nsbz: no spray buffer zone; vfs: vegetated filter strip; - not required as risk assessment already passes

Table CP 10.2-15 Aquatic organisms: acceptability of risk (PEC/RAC <1) for spiroxamine based on FOCUS Step 4 calculations for application of Prothioconazole + Spiroxamine EC 460 to Winter cereals (1 x 1.25 L/ha; early application)

Group		Fish chronic	Algae (Freshwater)	Algae (Marine)	Mesocosm
Test species		*Danio rerio*	*Scenedesmus subspicatus*	*Skeletonema costatum*	Algae and invertebrates
Endpoint (µg a.s./L)		EC_{10} 1.88	E_rC_{50} 12.0	E_rC_{50} 6.3	NOEC 1.0
AF		10	10	10	2
RAC (µg a.s./L)		0.188	1.2	0.63	0.5
FOCUS Scenario	$PEC_{sw\ max}$ (µg a.s./L)	PEC/RAC ratios			
Step 4 (20 m nsbz and 20 m vfs)					
D1 Ditch	0.200	**1.06**	0.167	0.317	0.400
D1 Stream	0.190	**1.01**	0.158	0.302	0.380
D2 Ditch	0.244	**1.30**	0.203	0.387	0.488
D2 Stream	0.213	**1.13**	0.178	0.338	0.426
D3 Ditch	0.179	0.952	0.149	0.284	0.358
D4 Pond	0.046	-	-	-	-
D4 Stream	0.179	0.952	0.149	0.284	0.358
D5 Pond	0.046	-	-	-	-
D5 Stream	0.193	**1.03**	0.161	0.306	0.386
D6 Ditch	0.174	0.926	0.145	0.276	0.348
R1 Pond	0.046	-	-	-	-
R1 Stream	0.165	0.878	0.138	0.262	0.330
R3 Stream	0.229	**1.22**	0.191	0.363	0.458
R4 Stream	0.171	0.910	0.143	0.271	0.342
Step 4 (30 m nsbz and 20 m vfs)					

Group		Fish chronic	Algae (Freshwater)	Algae (Marine)	Mesocosm
Test species		*Danio rerio*	*Scenedesmus subspicatus*	*Skeletonema costatum*	Algae and invertebrates
D1 Ditch	0.160	0.851	-	-	-
D1 Stream	0.130	0.691	-	-	-
D2 Ditch	0.192	**1.02**	-	-	-
D2 Stream	0.144	0.766	-	-	-
D3 Ditch	0.133	-	-	-	-
D4 Pond	0.038	-	-	-	-
D4 Stream	0.122	-	-	-	-
D5 Pond	0.038	-	-	-	-
D5 Stream	0.132	0.702	-	-	-
D6 Ditch	0.118	-	-	-	-
R1 Pond	0.038	-	-	-	-
R1 Stream	0.115	-	-	-	-
R3 Stream	0.159	0.846	-	-	-
R4 Stream	0.171	-	-	-	-

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the trigger of 1 are highlighted in **bold**; nsbz: no spray buffer zone; vfs: vegetated filter strip; - not required as risk assessment already passes

Table CP 10.2-16 Aquatic organisms: acceptability of risk (PEC/RAC <1) for spiroxamine based on FOCUS Step 4 calculations for application of Prothioconazole + Spiroxamine EC 460 to Winter cereals (1 x 1.25 L/ha; late application)

Group		Fish chronic	Algae (Freshwater)	Algae (Marine)	Mesocosm
Test species		*Danio rerio*	*Scenedesmus subspicatus*	*Skeletonema costatum*	Algae and invertebrates
Endpoint (µg a.s./L)		EC_{10} 1.88	E_rC_{50} 12.0	E_rC_{50} 6.3	NOEC 1.0
AF		10	10	10	2
RAC (µg a.s./L)		0.188	1.2	0.63	0.5
FOCUS Scenario	PEC$_{sw-max}$ (µg a.s./L)	PEC/RAC ratios			
Step 4 (20 m nsbz and 20 m vfs)					
D1 Ditch	0.244	**1.30**	0.203	0.387	0.488
D1 Stream	0.211	**1.12**	0.176	0.335	0.422
D2 Ditch	0.244	**1.30**	0.203	0.387	0.488
D2 Stream	0.214	**1.14**	0.178	0.340	0.428

Group		Fish chronic	Algae (Freshwater)	Algae (Marine)	Mesocosm
Test species		*Danio rerio*	*Scenedesmus subspicatus*	*Skeletonema costatum*	Algae and invertebrates
D3 Ditch	0.205	**1.09**	0.171	0.325	0.410
D4 Pond	0.046	-	-	-	-
D4 Stream	0.209	**1.11**	0.174	0.332	0.418
D5 Pond	0.046	-	-	-	-
D5 Stream	0.224	**1.19**	0.187	0.356	0.448
D6 Ditch	0.243	**1.29**	0.203	0.386	0.486
R1 Pond	0.057	-	-	-	-
R1 Stream	0.166	0.883	0.138	0.263	0.332
R3 Stream	0.229	**1.22**	0.191	0.363	0.458
R4 Stream	0.166	0.883	0.138	0.263	0.332
Step 4 (30 m nsbz and 20 m vfs)					
D1 Ditch	0.192	**1.02**	-	-	-
D1 Stream	0.143	0.761	-	-	-
D2 Ditch	0.192	**1.02**	-	-	-
D2 Stream	0.146	0.777	-	-	-
D3 Ditch	0.163	0.867	-	-	-
D4 Pond	0.038	-	-	-	-
D4 Stream	0.145	0.771	-	-	-
D5 Pond	0.038	-	-	-	-
D5 Stream	0.155	0.824	-	-	-
D6 Ditch	0.192	**1.02**	-	-	-
R1 Pond	0.051	-	-	-	-
R1 Stream	0.133	-	-	-	-
R3 Stream	0.160	0.851	-	-	-
R4 Stream	0.163	-	-	-	-

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the trigger of 1 are highlighted in **bold**; nsbz: no spray buffer zone; vfs: vegetated filter strip; - not required as risk assessment already passes

Table CP 10.2-17 Aquatic organisms: acceptability of risk (PEC/RAC <1) for spiroxamine based on FOCUS Step 4 calculations for application of Prothioconazole + Spiroxamine EC 460 to Spring cereals (2 x 1.25 L/ha; early application)

Group	Fish chronic	Algae (Freshwater)	Algae (Marine)	Mesocosm
Test species	*Danio rerio*	*Scenedesmus subspicatus*	*Skeletonema costatum*	Algae and invertebrates
Endpoint	EC_{10}	E_rC_{50}	E_rC_{50}	NOEC

Group		Fish chronic	Algae (Freshwater)	Algae (Marine)	Mesocosm
Test species		*Danio rerio*	*Scenedesmus subspicatus*	*Skeletonema costatum*	Algae and invertebrates
(µg a.s./L)		1.88	12.0	6.3	1.0
AF		10	10	10	2
RAC (µg a.s./L)		0.188	1.2	0.63	0.5
FOCUS Scenario	PEC $_{sw-max}$ (µg a.s./L)	PEC/RAC ratios			
Step 4 (20 m nsbz and 20 m vfs)					
D1 Ditch	0.297	**1.58**	0.248	0.471	0.594
D1 Stream	0.211	**1.12**	0.176	0.335	0.422
D3 Ditch	0.182	0.968	0.152	0.289	0.364
D4 Pond	0.065	-	-	-	-
D4 Stream	0.200	**1.06**	0.167	0.317	0.400
D5 Pond	0.062	-	-	-	-
D5 Stream	0.205	**1.09**	0.171	0.325	0.410
R4 Stream	0.372	**1.98**	0.310	0.590	0.744
Step 4 (30 m nsbz and 20 m vfs)					
D1 Ditch	0.236	**1.26**	-	-	-
D1 Stream	0.143	0.761	-	-	-
D3 Ditch	0.138	-	-	-	-
D4 Pond	0.054	-	-	-	-
D4 Stream	0.138	0.734	-	-	-
D5 Pond	0.051	-	-	-	-
D5 Stream	0.140	0.745	-	-	-
R4 Stream	0.372	**1.98**	-	-	-

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the trigger of 1 are highlighted in **bold**; nsbz: no spray buffer zone; vfs: vegetated filter strip; - not required as risk assessment already passes

Table CP 10.2.18 Aquatic organisms: acceptability of risk (PEC/RAC <1) for spiroxamine based on FOCUS Step 4 calculations for application of Prothioconazole + Spiroxamine EC 460 to Spring cereals (2 x 1.25 L/ha; late application)

Group	Fish chronic	Algae (Freshwater)	Algae (Marine)	Mesocosm
Test species	*Danio rerio*	*Scenedesmus subspicatus*	*Skeletonema costatum*	Algae and invertebrates
Endpoint	EC_{10}	E_rC_{50}	E_rC_{50}	NOEC
(µg a.s./L)	1.88	12.0	6.3	1.0

Group		Fish chronic	Algae (Freshwater)	Algae (Marine)	Mesocosm
Test species		*Danio rerio*	*Scenedesmus subspicatus*	*Skeletonema costatum*	Algae and invertebrates
AF		10	10	10	2
RAC (µg a.s./L)		0.188	1.2	0.63	0.5
FOCUS Scenario	PEC $_{sw-max}$ (µg a.s./L)	PEC/RAC ratios			
Step 4 (20 m nsbz and 20 m vfs)					
D1 Ditch	0.275	**1.46**	0.229	0.437	0.550
D1 Stream	0.211	**1.12**	0.176	0.335	0.422
D3 Ditch	0.194	**1.03**	0.162	0.308	0.388
D4 Pond	0.066	-			
D4 Stream	0.210	**1.12**	0.175	0.333	0.420
D5 Pond	0.066	-			-
D5 Stream	0.224	**1.19**	0.187	0.356	0.448
R4 Stream	0.227	**1.21**	0.189	0.360	0.454
Step 4 (30 m nsbz and 20 m vfs)					
D1 Ditch	0.219	**1.16**	-	-	-
D1 Stream	0.143	0.761		-	-
D3 Ditch	0.154	0.819	-	-	-
D4 Pond	0.055	-		-	-
D4 Stream	0.145	0.771	-	-	-
D5 Pond	0.055	-	-	-	-
D5 Stream	0.155	0.824			-
R4 Stream	0.227	**1.21**	-	-	-

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the trigger of 1 are highlighted in **bold**; nsbz: no spray buffer zone; vfs: vegetated filter strip; - not required as risk assessment already passes

Table CP 10.2-19 Aquatic organisms: acceptability of risk (PEC/RAC <1) for spiroxamine based on FOCUS Step 4 calculations for application of Prothioconazole + Spiroxamine EC 460 to Winter cereals (2 x 1.25 L/ha; early application)

Group	Fish chronic	Algae (Freshwater)	Algae (Marine)	Mesocosm
Test species	*Danio rerio*	*Scenedesmus subspicatus*	*Skeletonema costatum*	Algae and invertebrates
Endpoint (µg a.s./L)	EC_{10} 1.88	E_rC_{50} 12.0	E_rC_{50} 6.3	NOEC 1.0
AF	10	10	10	2

Group		Fish chronic	Algae (Freshwater)	Algae (Marine)	Mesocosm
Test species		*Danio rerio*	*Scenedesmus subspicatus*	*Skeletonema costatum*	Algae and invertebrates
RAC (µg a.s./L)		0.188	1.2	0.63	0.5
FOCUS Scenario	PEC $_{sw-max}$ (µg a.s./L)	PEC/RAC ratios			
Step 4 (20 m nsbz and 20 m vfs)					
D1 Ditch	0.208	**1.11**	0.173	0.330	0.416
D1 Stream	0.190	**1.01**	0.158	0.302	0.380
D2 Ditch	0.244	**1.30**	0.203	0.387	0.488
D2 Stream	0.213	**1.13**	0.178	0.338	0.426
D3 Ditch	0.179	0.952	0.149	0.284	0.358
D4 Pond	0.058	-	-	-	-
D4 Stream	0.179	0.952	0.149	0.284	0.358
D5 Pond	0.066	-	-	-	-
D5 Stream	0.193	**1.03**	0.161	0.306	0.386
D6 Ditch	0.193	**1.03**	0.161	0.306	0.386
R1 Pond	0.065	-	-	-	-
R1 Stream	0.239	**1.27**	0.199	0.379	0.478
R3 Stream	0.237	**1.26**	0.198	0.376	0.474
R4 Stream	0.397	**2.11**	0.331	0.630	0.794
Step 4 (30 m nsbz and 20 m vfs)					
D1 Ditch	0.167	0.888	-	-	-
D1 Stream	0.130	0.691	-	-	-
D2 Ditch	0.192	**1.02**	-	-	-
D2 Stream	0.144	0.766	-	-	-
D3 Ditch	0.133	-	-	-	-
D4 Pond	0.049	-	-	-	-
D4 Stream	0.122	-	-	-	-
D5 Pond	0.055	-	-	-	-
D5 Stream	0.132	0.702	-	-	-
D6 Ditch	0.156	0.830	-	-	-
R1 Pond	0.057	-	-	-	-
R1 Stream	0.239	**1.27**	-	-	-
R3 Stream	0.237	**1.26**	-	-	-
R4 Stream	0.397	**2.11**	-	-	-

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the trigger of 1 are highlighted in **bold**; nsbz: no spray buffer zone; vfs: vegetated filter strip; - not required as risk assessment already passes

Table CP 10.2-20 Aquatic organisms: acceptability of risk (PEC/RAC <1) for spiroxamine based on FOCUS Step 4 calculations for application of Prothioconazole + Spiroxamine EC 460 to Winter cereals (2 x 1.25 L/ha; late application)

Group		Fish chronic	Algae (Freshwater)	Algae (Marine)	Mesocosm
Test species		*Danio rerio*	*Scenedesmus subspicatus*	*Skeletonema costatum*	Algae and invertebrates
Endpoint (µg a.s./L)		EC_{10} 1.88	E_rC_{50} 12.0	E_rC_{50} 6.3	NOEC 1.0
AF		10	10	10	2
RAC (µg a.s./L)		0.188	1.2	0.63	0.5
FOCUS Scenario	PEC $_{sw-max}$ (µg a.s./L)	PEC/RAC ratios			
Step 4 (20 m nsbz and 20 m vfs)					
D1 Ditch	0.299	**1.59**	0.249	0.475	0.598
D1 Stream	0.211	**1.12**	0.176	0.335	0.422
D2 Ditch	0.303	**1.61**	0.253	0.481	0.606
D2 Stream	0.253	**1.35**	0.211	0.402	0.506
D3 Ditch	0.205	**1.09**	0.171	0.325	0.410
D4 Pond	0.066	-	-	-	-
D4 Stream	0.209	**1.11**	0.174	0.332	0.418
D5 Pond	0.066	-	-	-	-
D5 Stream	0.224	**1.19**	0.187	0.356	0.448
D6 Ditch	0.243	**1.29**	0.203	0.386	0.486
R1 Pond	0.092	0.489	-	-	-
R1 Stream	0.241	**1.28**	0.201	0.383	0.482
R3 Stream	0.229	**1.22**	0.191	0.363	0.458
R4 Stream	0.166	0.883	0.138	0.263	0.332
Step 4 (30 m nsbz and 20 m vfs)					
D1 Ditch	0.239	**1.27**	-	-	-
D1 Stream	0.143	0.761	-	-	-
D2 Ditch	0.241	**1.28**	-	-	-
D2 Stream	0.174	0.926	-	-	-
D3 Ditch	0.163	0.867	-	-	-
D4 Pond	0.055	-	-	-	-

Group		Fish chronic	Algae (Freshwater)	Algae (Marine)	Mesocosm
Test species		*Danio rerio*	*Scenedesmus subspicatus*	*Skeletonema costatum*	Algae and invertebrates
D4 Stream	0.145	0.771	-	-	-
D5 Pond	0.055	-	-	-	-
D5 Stream	0.155	0.824	-	-	-
D6 Ditch	0.192	**1.02**	-	-	-
R1 Pond	0.084	-	-	-	-
R1 Stream	0.241	**1.28**	-	-	-
R3 Stream	0.160	0.851	-	-	-
R4 Stream	0.165	-	-	-	-

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the trigger of 1 are highlighted in **bold**; nsbz: no spray buffer zone; vfs: vegetated filter strip; - not required as risk assessment already passes

For the algal risk assessments, including those organisms covered by the mesocosm study, an acceptable risk from exposure to spiroxamine can be concluded for all proposed uses of Prothioconazole + Spiroxamine EC 460 to cereals when mitigation in the form of a 20 m no-spray buffer zone with a 20 m vegetated filter strip is applied.

For the chronic fish risk assessment, the PEC/RAC ratios for majority of FOCUS scenarios are <1 when up to 95% total application mitigation in the form of a 30 m no-spray buffer zone with a 20 m vegetated filter strip was applied, thereby demonstrating an acceptable chronic risk to fish for the majority of scenarios.

However, for some scenarios further refinement of the chronic fish risk assessment is required and has been discussed at the end of this section.

It is noted that for the single application of 1.25 L product/ha to winter cereals (early and late applications) and for early applications to Spring cereals, the highest PEC/RAC ratio was only 1.02 when mitigation in the form of a 30 m no-spray buffer zone with a 20 m vegetated filter strip was applied. Given this was a single value for one scenario, in practical terms this can be considered to demonstrate acceptable risks to the relevant FOCUS scenarios for these uses.

The table below provides a summary of those FOCUS scenarios for which an acceptable risk can be demonstrated using up to 95% total application mitigation and those scenarios for which further refinement is required. For all uses, this risk assessment shows acceptable risk for the majority scenarios and there are relatively few scenarios where further refinement is required.

Table CP 10.2-21 Summary of aquatic risk assessment: FOCUS scenarios with an acceptable risk demonstrated and those requiring further refinement

Proposed use of Prothioconazole + Spiroxamine EC 460 to cereals (L/ha)		FOCUS scenarios for which acceptable risks have been demonstrated using a 30 m nsbz with a 20 m vfs	FOCUS scenarios for which further refinement is required
1 x 1.25 Spring cereals	Early	D1 Stream, D3 Ditch, D4 Pond*, D4 Stream, D5 Pond*, D5 Stream, R4 Stream	D1 Ditch
	Late	D1 Stream, D3 Ditch, D4 Pond*, D4 Stream, D5 Pond*, D5 Stream	D1 Ditch, R4 Stream

Proposed use of Prothioconazole + Spiroxamine EC 460 to cereals (L/ha)		FOCUS scenarios for which acceptable risks have been demonstrated using a 30 m nsbz with a 20 m vfs	FOCUS scenarios for which further refinement is required
1 x 1.25 Winter cereals	Early	D1 Ditch, D1 Stream, D2 Stream, D3 Ditch, D4 Pond*, D4 Stream, D5 Pond*, D5 Stream, D6 Ditch, R1 Pond*, R1 Stream, R3 Stream, R4 Stream	D2 Ditch
	Late	D1 Stream, D2 Stream, D3 Ditch, D4 Pond*, D4 Stream, D5 Pond*, D5 Stream, R1 Pond*, R1 Stream, R3 Stream, R4 Stream	D1 Ditch, D2 Ditch, D6 Ditch
2 x 1.25 Spring cereals	Early	D1 Stream, D3 Ditch, D4 Pond*, D4 Stream, D5 Pond*, D5 Stream,	D1 Ditch, R4 Stream
	Late	D1 Stream, D3 Ditch, D4 Pond*, D4 Stream, D5 Pond*, D5 Stream,	D1 Ditch, R4 Stream
2 x 1.25 Winter cereals	Early	D1 Ditch, D1 Stream, D2 Stream, D3 Ditch, D4 Pond*, D4 Stream, D5 Pond*, D5 Stream, D6 Ditch, R1 Pond*	D2 Ditch, R4 Stream, R3 Stream, R4 Stream
	Late	D1 Stream, D2 Stream, D3 Ditch, D4 Pond*, D4 Stream, D5 Pond*, D5 Stream, R1 Pond, R3 Stream, R4 Stream	D1 Ditch, D2 Ditch, D6 Ditch, R1 Stream,

nsbz: no spray buffer zone; vfs: vegetated filter strip
* Scenario for this use passes the risk assessment at Step 3 therefore no mitigation required

Metabolites of spiroxamine

Risk assessments for the metabolites of spiroxamine; KWG 4168-desethyl (M01), KWG 4168-despropyl (M02), KWG 4169-N-oxide (M03) and KWG 4168-acid (M06) have been presented below. As for spiroxamine, early and late applications at 1 x 1.25 L/ha and 2 x 1.25 L/ha to Spring and Winter cereals have been considered in the risk assessments.

The selection of endpoints for the metabolite risk assessment have been discussed at the start of Section 10.2. As previously discussed, to account for possible selective isomeric degradation, Uncertainty Factors (UF) have been applied to the RAC values (refer to Table CP 10.2-4).

Table CP 10.2-22 Aquatic organisms: acceptability of risk (PEC/RAC <1) for M01 and M02 based on FOCUS Steps 1 and 2 calculations for application of Prothioconazole + Spiroxamine EC 460 to Spring & Winter cereals (1 x 1.25 L/ha; early & late application)

Metabolite	M01			M02		
Group	**Fish acute**	**Invertebrate acute**	**Algae**	**Fish acute**	**Invertebrate acute**	**Algae**
Test species	***Danio rerio***	***Daphnia magna***	***Desmodesmus subspicatus***	***Danio rerio***	***Daphnia magna***	***Pseudokirchneriella subcapitata***
Endpoint (µg/L)	LC_{50} 2410 [a]	EC_{50} 3000 [a]	E_rC_{50} 737	LC_{50} 2410 [a]	EC_{50} 3000 [a]	E_rC_{50} 383
AF	100	100	10	100	100	10

Metabolite	**M01**			**M02**		
Group	**Fish acute**	**Invertebrate acute**	**Algae**	**Fish acute**	**Invertebrate acute**	**Algae**
Test species	***Danio rerio***	***Daphnia magna***	***Desmodesmus subspicatus***	***Danio rerio***	***Daphnia magna***	***Pseudokirchneriella subcapitata***
RAC (µg/L)	24.1	30.0	73.7	24.1	30.0	38.3
UF	4.76	4.76	4.76	12.5	12.5	12.5
Corrected RAC (µg/L)	5.06	6.30	15.5	1.93	2.40	3.06
FOCUS Scenario	PEC/RAC ratios					
Step 1	PEC $_{sw-max}$ 7.151 µg/L			PEC $_{sw-max}$ 5.983 µg/L		
	1.41	**1.13**	0.462	**3.10**	**2.49**	**1.95**
Step 2						
NEU	PEC $_{sw-max}$ 0.236 µg/L			PEC $_{sw-max}$ 0.198 µg/L		
	0.0466	0.0374	-	0.103	0.0825	0.0646
SEU	PEC $_{sw-max}$ 0.436 µg/L			PEC $_{sw-max}$ 0.367 µg/L		
	0.0861	0.0692	-	0.190	0.153	0.120

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; UF: Uncertainty Factor
[a] Endpoint has assumed equivalent toxicity of the parent material; PEC/RAC ratios above the trigger of 1 are highlighted in **bold**; - not required as risk assessment passes using Step 1 PEC values

Table CP 10.2-23 Aquatic organisms: acceptability of risk (PEC/RAC <1) for M03 and M06 based on FOCUS Steps 1 and 2 calculations for application of Prothioconazole + Spiroxamine EC 460 to Spring & Winter cereals (1 x 1.25 L/ha; early & late application)

Metabolite	**M03**			**M06**		
Group	**Fish acute**	**Invertebrate acute**	**Algae**	**Fish acute**	**Invertebrate acute**	**Algae**
Test species	***Danio rerio***	***Daphnia magna***	***Desmodesmus subspicatus***	***Danio rerio***	***Daphnia magna***	***Desmodesmus subspicatus***
Endpoint (µg/L)	LC_{50} 2410 [a]	EC_{50} >100000	E_rC_{50} 31700	LC_{50} 2410 [a]	EC_{50} 3000 [a]	E_rC_{50} 3200
AF	100	100	10	100	100	10
RAC (µg/L)	24.1	1000	3170	24.1	30.0	320
UF	10.0	10.0	10.0	2.32	2.32	2.32
Corrected RAC (µg/L)	2.41	100	317	10.4	12.9	138
FOCUS Scenario	PEC/RAC ratios					
Step 1	PEC $_{sw-max}$ 16.452 µg/L			PEC $_{sw-max}$ 139.851 µg/L		

Metabolite	M03			M06		
Group	Fish acute	Invertebrate acute	Algae	Fish acute	Invertebrate acute	Algae
Test species	*Danio rerio*	*Daphnia magna*	*Desmodesmus subspicatus*	*Danio rerio*	*Daphnia magna*	*Desmodesmus subspicatus*
	6.83	0.165	0.0519	**13.5**	**10.8**	**1.01**
Step 2						
NEU	PEC $_{sw-max}$ 0.606 µg/L			PEC $_{sw-max}$ 5.536 µg/L		
	0.251	-	-	0.533	0.428	0.0401
SEU	PEC $_{sw-max}$ 1.047 µg/L			PEC $_{sw-max}$ 9.403 µg/L		
	0.434	-	-	0.905	0.727	0.0682

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; UF: Uncertainty Factor

[a] Endpoint has assumed equivalent toxicity of the parent material; PEC/RAC ratios above the trigger of 1 are highlighted in **bold**; - not required as risk assessment passes using Step 1 PEC values

Table CP 10.2-24 Aquatic organisms: acceptability of risk (PEC/RAC <1) for M01 and M02 based on FOCUS Steps 1 and 2 calculations for application of Prothioconazole+Spiroxamine EC 460 to Spring & Winter cereals (2 x 1.25 L/ha; early & late application)

Metabolite	M01			M02		
Group	Fish acute	Invertebrate acute	Algae	Fish acute	Invertebrate acute	Algae
Test species	*Danio rerio*	*Daphnia magna*	*Desmodesmus subspicatus*	*Danio rerio*	*Daphnia magna*	*Pseudokirchneriella subcapitata*
Endpoint (µg/L)	LC_{50} 2410 [a]	EC_{50} 3000 [a]	E_rC_{50} 737	LC_{50} 2410 [a]	EC_{50} 3000 [a]	E_rC_{50} 383
AF	100	100	10	100	100	10
RAC (µg/L)	24.1	30.0	73.7	24.1	30.0	38.3
UF	4.76	4.76	4.76	12.5	12.5	12.5
Corrected RAC (µg/L)	5.06	6.30	15.5	1.93	2.40	3.06
FOCUS Scenario	PEC/RAC ratios					
Step 1	PEC $_{sw-max}$ 7.151 µg/L			PEC $_{sw-max}$ 5.983 µg/L		
	1.41	**1.13**	0.462	**3.10**	**2.49**	**1.95**

Metabolite	M01			M02		
Group	Fish acute	Invertebrate acute	Algae	Fish acute	Invertebrate acute	Algae
Test species	*Danio rerio*	*Daphnia magna*	*Desmodesmus subspicatus*	*Danio rerio*	*Daphnia magna*	*Pseudokirchneriella subcapitata*
Step 2						
NEU	PEC $_{sw-max}$ 0.444 µg/L			PEC $_{sw-max}$ 0.374 µg/L		
	0.0877	0.0704	-	0.194	0.156	0.122
SEU	PEC $_{sw-max}$ 0.826 µg/L			PEC $_{sw-max}$ 0.699 µg/L		
	0.163	0.131	-	0.363	0.291	0.228

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; UF: Uncertainty Factor
[a] Endpoint has assumed equivalent toxicity of the parent material; PEC/RAC ratios above the trigger of 1 are highlighted in **bold**; - not required as risk assessment passed using Step 1 PEC values

Table CP 10.2-25 Aquatic organisms: acceptability of risk (PEC/RAC <1) for M03 and M06 based on FOCUS Steps 1 and 2 calculations for application of Prothioconazole + Spiroxamine EC 460 to Spring & Winter cereals (2 x 1.25 L/ha; early & late application)

Metabolite	M03			M06		
Group	Fish acute	Invertebrate acute	Algae	Fish acute	Invertebrate acute	Algae
Test species	*Danio rerio*	*Daphnia magna*	*Desmodesmus subspicatus*	*Danio rerio*	*Daphnia magna*	*Desmodesmus subspicatus*
Endpoint (µg/L)	LC_{50} 2410 [a]	EC_{50} >100000	E_rC_{50} 31700	LC_{50} 2410 [a]	EC_{50} 3000 [a]	E_rC_{50} 3200
AF	100	100	10	100	100	10
RAC (µg/L)	24.1	1000	3170	24.1	30.0	320
UF	10.0	10.0	10.0	2.32	2.32	2.32
Corrected RAC (µg/L)	2.41	100	317	10.4	12.9	138
FOCUS Scenario	PEC/RAC ratios					
Step 1	PEC $_{sw-max}$ 16.452 µg/L			PEC $_{sw-max}$ 139.851 µg/L		
	6.83	0.165	0.0519	**13.5**	**10.8**	**1.01**
Step 2						
NEU	PEC $_{sw-max}$ 1.086 µg/L			PEC $_{sw-max}$ 9.945 µg/L		
	0.451	-	-	0.957	0.769	0.0721
SEU	PEC $_{sw-max}$ 1.882 µg/L			PEC $_{sw-max}$ 16.988 µg/L		
	0.781	-	-	**1.64**	**1.31**	0.123

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; UF: Uncertainty Factor

[a] Endpoint has assumed equivalent toxicity of the parent material; PEC/RAC ratios above the trigger of 1 are highlighted in **bold**; - not required as risk assessment passes using Step 1 PEC values

For KWG 4168-desethyl (M01), KWG 4168-despropyl (M02) and KWG 4169-N-oxide (M03) acceptable risks to aquatic organisms have been demonstrated using either FOCUS Step 1 or Step 2 PEC_{sw} values for all proposed uses of Prothioconazole + Spiroxamine EC 460 on cereals.

For KWG 4168-acid (M06) acceptable risks to aquatic organisms have been demonstrated for the proposed uses of Prothioconazole + Spiroxamine EC 460 on Spring and Winter cereals (early and late applications) at 1 x 1.25 L/ha. However, for the proposed uses at 2 x 1.25 L/ha, possible risks to aquatic organisms have been identified. Further environmental fate data for this metabolite are currently being generated and the PEC_{sw} modelling will be updated and submitted as part of the top-up submission for the Renewal of Approval of spiroxamine.

Prothioconazole

Risk assessments for prothioconazole and the metabolites prothioconazole-desthio, prothioconazole-S-methyl and 1,2,4-Triazole have been presented below. As already discussed at the beginning of Section 10.2, the toxicity endpoints have been taken directly from the EFSA Conclusion for prothioconazole (EFSA Scientific Report (2007) 106, 1-98) without further consideration. PEC_{sw} values for prothioconazole and metabolites have been taken from the current draft RAR for spiroxamine (Spiroxamine dRAR, Volume 3, Annex B.9) and are considered to cover the proposed uses on cereals being assessed here.

Table CP 10.2-26
Aquatic organisms: acceptability of risk (PEC/RAC <1) for prothioconazole for each aquatic group based on FOCUS Step 2 calculations for application of prothioconazole + Spiroxamine EC 460 to Spring and Winter cereals at 1.25 L/ha

Group		Fish acute	Fish chronic	Invertebrate acute	Invertebrate chronic	Algae	Sediment dweller
Test species		*Oncorhynchus mykiss*	*Oncorhynchus mykiss*	*Daphnia magna*	*Daphnia magna*	*Pseudokirchneriella subcapitata*	*Chironomus riparius*
Endpoint (µg a.s./L)		LC_{50} 1830	NOEC 308	EC_{50} 1300	NOEC 560	E_rC_{50} 2180	NOEC 9140
AF		100	10	100	10	10	10
RAC (µg/L)		18.3	30.8	13	56	218	914
FOCUS Scenario	PEC_{sw-max} (µg a.s./L)	PEC/RAC ratios					
Step 2							
NEU	2.048	0.112	0.0665	0.158	0.0366	0.00939	0.00224
SEU	2.048	0.112	0.0665	0.158	0.0366	0.00939	0.00224

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration

The PEC/RAC ratios for all organism groups are <1 using the FOCUS Step 2 PEC_{sw} value of 2.048 µg a.s./L. Thus, there are no unacceptable risks to aquatic organisms from exposure to prothioconazole following applications of Prothioconazole + Spiroxamine EC 460 to cereals at 1.25 L/ha.

A risk assessment for the metabolite prothioconazole-desthio has been presented below for Spring cereals and Winter cereals, respectively.

Table CP 10.2-27 Aquatic organisms: acceptability of risk (PEC/RAC <1) for Prothioconazole-desthio for each aquatic group based on FOCUS Step 2, 3 and 4 calculations for application of prothioconazole + Spiroxamine EC 460 to Spring cereals at 1.25 L/ha

Group		Fish acute	Fish chronic	Invertebrate acute	Invertebrate chronic	Algae	Sediment dweller
Test species		*Oncorhyn-chus mykiss*	*Oncorhyn-chus mykiss*	*Daphnia magna*	*Daphnia magna*	*Scenedesmus subspicatus*	*Chironomus riparius*
Endpoint (µg/L)		LC_{50} 6630	NOEC 3.34	EC_{50} 10000	NOEC 100	E_rC_{50} 550	NOEC 2000
AF		100	10	100	10	10	10
RAC (µg/L)		66.3	0.334	100	10	55	200
FOCUS Scenario	PEC_{sw-max} (µg/L)	PEC/RAC ratios					
Step 2							
NEU	4.138	0.0624	**12.4**	0.0414	0.414	0.0752	0.0207
SEU	7.361	0.111	**22.0**	0.0736	0.736	0.134	0.0368
Step 3							
D1 ditch	0.483	-	**1.45**	-	-	-	-
D1 stream	0.278	-	0.832	-	-	-	-
D3 ditch	0.318	-	0.952	-	-	-	-
D4 pond	0.017	-	0.0509	-	-	-	-
D4 stream	0.271	-	0.811	-	-	-	-
D5 pond	0.017	-	0.0509	-	-	-	-
D5 stream	0.274	-	0.820	-	-	-	-
R4 stream	0.736	-	**2.20**	-	-	-	-
Step 4 (5 m no-spray buffer zone)							
D1 ditch	0.124	-	0.371	-	-	-	-
D1 stream	0.098	-	-	-	-	-	-
D3 ditch	0.083	-	-	-	-	-	-
D4 pond	0.015	-	-	-	-	-	-
D4 stream	0.086	-	-	-	-	-	-
D5 pond	0.015	-	-	-	-	-	-
D5 stream	0.097	-	-	-	-	-	-
R4 stream	0.736	-	**2.20**	-	-	-	-
Step 4 (10 m no-spray buffer zone; 80/95% reduction)							
R4 stream	0.176	-	0.527	-	-	-	-

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the trigger of 1 are highlighted in **bold**

Table CP 10.2-28 Aquatic organisms: acceptability of risk (PEC/RAC <1) for Prothioconazole-desthio for each aquatic group based on FOCUS Step 2, 3 and 4 calculations for application of prothioconazole + Spiroxamine EC 460 to Winter cereals at 1.25 L/ha

Group		Fish acute	Fish chronic	Invertebrate acute	Invertebrate chronic	Algae	Sediment dweller
Test species		*Oncorhynchus mykiss*	*Oncorhynchus mykiss*	*Daphnia magna*	*Daphnia magna*	*Scenedesmus subspicatus*	*Chironomus riparius*
Endpoint (µg/L)		LC_{50} 6630	NOEC 3.34	EC_{50} 10000	NOEC 100	E_rC_{50} 550	NOEC 2000
AF		100	10	100	10	10	10
RAC (µg/L)		66.3	0.334	100	10	55	200
FOCUS Scenario	PEC $_{sw-max}$ (µg/L)	PEC/RAC ratios					
Step 2							
NEU	4.138	0.0624	**12.4**	0.0414	0.414	0.0752	0.0207
SEU	7.361	0.111	**22.0**	0.0736	0.736	0.134	0.0368
Step 3							
D1 ditch	0.482	-	**1.44**	-	-	-	-
D1 stream	0.278	-	0.832	-	-	-	-
D2 ditch	0.326	-	0.976	-	-	-	-
D2 stream	0.283	-	0.847	-	-	-	-
D3 ditch	0.318	-	0.952	-	-	-	-
D4 pond	0.017	-	0.0509	-	-	-	-
D4 stream	0.271	-	0.811	-	-	-	-
D5 pond	0.017	-	0.0509	-	-	-	-
D5 stream	0.275	-	0.823	-	-	-	-
D6 ditch	0.319	-	0.955	-	-	-	-
R1 pond	0.111	-	0.332	-	-	-	-
R1 stream	1.098	-	**3.29**	-	-	-	-
R3 stream	1.104	-	**3.31**	-	-	-	-
R4 stream	1.244	-	**3.72**	-	-	-	-
Step 4 (5 m no-spray buffer zone)							
D1 ditch	0.124		0.371	-	-	-	-
D1 stream	0.098	-	-	-	-	-	-
D2 ditch	0.086	-	-	-	-	-	-
D2 stream	0.100	-	-	-	-	-	-
D3 ditch	0.083	-	-	-	-	-	-

Group		Fish acute	Fish chronic	Invertebrate acute	Invertebrate chronic	Algae	Sediment dweller
Test species		*Oncorhynchus mykiss*	*Oncorhynchus mykiss*	*Daphnia magna*	*Daphnia magna*	*Scenedesmus subspicatus*	*Chironomus riparius*
D4 pond	0.014	-	-	-	-	-	-
D4 stream	0.096	-	-	-	-	-	-
D5 pond	0.015	-	-	-	-	-	-
D5 stream	0.097	-	-	-	-	-	-
D6 ditch	0.083	-	-	-	-	-	-
R1 pond	0.110	-	-	-	-	-	-
R1 stream	1.098	-	**3.29**	-	-	-	-
R3 stream	1.104	-	**3.31**	-	-	-	-
R4 stream	1.244	-	**3.72**	-	-	-	-
Step 4 (10 m no-spray buffer zone; 80/95% reduction)							
R1 stream	0.261	-	0.781	-	-	-	-
R3 stream	0.264	-	0.790	-	-	-	-
R4 stream	0.294	-	0.880	-	-	-	-

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the trigger of 1 are highlighted in **bold**

For both Spring and Winter cereals, acceptable risks from exposure to prothioconazole-desthio, following Application of Prothioconazole + Spiroxamine EC 460, can be demonstrated (PEC/RAC ratios <1) using FOCUS Step 2 PEC_{sw} values for all organism groups with the exception of the chronic fish risk assessment. For the chronic fish risk assessment, the majority of relevant FOCUS scenarios passed the risk assessment using Step 3 PEC_{sw} values but refinement by the use of Step 4 PEC_{sw} values was necessary for some scenarios. The chronic risks to fish from exposure to prothioconazole-desthio were demonstrated to be acceptable when application in the form of a 10 m no-spray buffer zone with run-off reduction in the form of a vegetated filter strip was applied. It should be noted that this mitigation is covered by the mitigation proposed based on the spiroxamine risk assessments.

A risk assessment for the metabolites prothioconazole-S-methyl and 1,2,4-Triazole have been presented below.

Table CP 10.2-29 Aquatic organisms: acceptability of risk (PEC/RAC <1) for prothioconazole-S-methyl and 1,2,4-Triazole for each aquatic group based on FOCUS Step 2 calculations for application of prothioconazole + Spiroxamine EC 460 to Spring and Winter cereals at 1.25 L/ha

Metabolite	Prothioconazole-S-methyl			1,2,4-Triazole			
Group	Fish acute	Invertebrate acute	Algae	Fish acute	Fish chronic	Invertebrate acute	Algae
Test species	*Oncorhynchus mykiss*	*Daphnia magna*	*Pseudokirchneriella subcapitata*	*Oncorhynchus mykiss*	*Oncorhynchus mykiss*	*Daphnia magna*	*Pseudokirchneriella subcapitata*
Endpoint (µg/L)	LC_{50} 1800	EC_{50} 2800	E_rC_{50} 47400	LC_{50} 498000	NOEC 3200	EC_{50} 900000	E_rC_{50} 22500

Metabolite	Prothioconazole-S-methyl			1,2,4-Triazole			
Group	Fish acute	Invertebrate acute	Algae	Fish acute	Fish chronic	Invertebrate acute	Algae
Test species	*Oncorhynchus mykiss*	*Daphnia magna*	*Pseudokirchneriella subcapitata*	*Oncorhynchus mykiss*	*Oncorhynchus mykiss*	*Daphnia magna*	*Pseudokirchneriella subcapitata*
AF	100	100	10	100	10	100	10
RAC (µg/L)	18	28	4740	4980	320	9000	2250
FOCUS Scenario	PEC/RAC ratios						
Step 2							
NEU	PEC $_{sw-max}$ 0.383 µg/L			PEC $_{sw-max}$ 0.262 µg/L			
	0.0212	0.0136	0.0000806	0.0000526	0.000819	0.0000291	0.000116
SEU	PEC $_{sw-max}$ 0.670 µg/L			PEC $_{sw-max}$ 0.262 µg/L			
	0.0372	0.0239	0.000141	0.0000526	0.000819	0.0000291	0.000116

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration

The PEC/RAC ratios for all organism groups are <1 using FOCUS Step 2 PEC$_{sw}$ values. Thus, there are no unacceptable risks to aquatic organisms from exposure to prothioconazole-S-methyl and 1,2,4-Triazole following applications of Prothioconazole + Spiroxamine EC 460 to cereals at 1.25 L/ha.

Formulation risk assessment

A formulation specific risk assessment using the available formulation data with Prothioconazole + Spiroxamine EC 460 has been conducted and presented below. Formulations are considered to remain intact only for very short periods following application therefore exposure due to spray drift only has been considered here. The maximum single application rate of 1.25 L/ha has been used in the risk assessment below and is considered to cover all proposed uses of Prothioconazole + Spiroxamine EC 460. Further details on the PEC$_{sw}$ calculations can be found in Document M-CP Section 9 Environmental Fate.

Table CP 10.2-30 Aquatic organisms; acceptability of risk (PEC/RAC <1) for Prothioconazole + Spiroxamine EC 460 based on spray drift PEC$_{sw}$ calculations for application of Prothioconazole + Spiroxamine EC 460 to cereals (2 x 1.25 L/ha)

Group		Fish acute	Invertebrate acute	Algae	Aquatic macrophytes
Test species		*Oncorhynchus mykiss*	*Daphnia magna*	*Pseudokirchneriella subcapitata*	*Lemna gibba*
Endpoint (µg/L)		LC_{50} 6570	EC_{50} 6300	E_rC_{50} 147	E_rC_{50} 54.2
AF		100	100	10	10
RAC (µg/L)		65.7	63	14.7	5.42
Waterbody type	PECsw (µg/L)	PEC/RAC ratios			
Default distance					
Ditch	7.902	0.120	0.125	0.538	**1.46**

Group		Fish acute	Invertebrate acute	Algae	Aquatic macrophytes
Test species		*Oncorhynchus mykiss*	*Daphnia magna*	*Pseudokirchneriella subcapitata*	*Lemna gibba*
Pond	0.2694	0.00410	0.00428	0.0183	0.0497
Stream	5.865	0.0893	0.0931	0.399	**1.08**
5 m distance					
Ditch	2.142	0.0326	0.0340	0.146	0.395
Pond	0.2332	0.00355	0.00370	0.0159	0.0430
Stream	2.142	0.0326	0.0340	0.146	0.395

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the trigger of 1 are highlighted in **bold**

At the default distance of 1 m, possible risks to aquatic organisms have been identified following application of Prothioconazole + Spiroxamine EC 460, however, acceptable risks to aquatic organisms from exposure to Prothioconazole + Spiroxamine EC 460 have been demonstrated when suitable mitigation is applied. For all proposed uses on cereals a 5 m no spray buffer zone needs to be applied as an application mitigation measure in order for the risks to aquatic organisms from Prothioconazole + Spiroxamine EC 460 exposure to be acceptable. It should be noted that the proposed mitigation measures for the risk assessment of spiroxamine also cover this mitigation.

Risk assessment for combined exposure of spiroxamine and prothioconazole

Mixture toxicity and exposure was calculated using the concentration addition model (CA model), according to Section 10.3 of the Aquatic Guidance Document[6], following the step-wise approach outlined in Section 10.3.11. Where there are multiple active substances present in a product, an assessment of the mixture toxicity and exposure should be made to determine whether the active substances act more (synergistically) or less (antagonistically) than expected by comparing the predicted toxicity with the measured toxicity.

The CA model is based on the following equation:

$$ECx_{mix-CA} = \left(\sum_{i=1}^{n} \frac{p_i}{ECx_i} \right)^{-1}$$

Where:

n	number of mixture components
i	index from 1…n mixture components
p_i	the i^{th} component as a relative fraction of the mixture composition
ECx_i	concentration of component i provoking x% effect (pragmatically, $NOEC_i$ may also be inserted too).

For a mixture containing two active substances this can be more simply expressed using the following equation:

[6] Guidance on the tiered risk assessment for edge-of-field surface waters. EFSA Journal 2013; 11(7):3290

$$EC_{50mix\text{-}CA} = 1 / (p^1/EC_{50}^1 + p^2/EC_{50}^2)$$

Where;
$EC_{50mix\text{-}CA}$: calculated mixture toxicity
[1] and [2] indicate active substance 1 and active substance 2, respectively
p: the proportion in the mix of each active as a fraction; Σp should always = 1
EC_{50}: experimentally determined EC_{50}/LC_{50}/NOEC. These should be based on the same measured parameter (*e.g.* growth rate or biomass)

For the mixture toxicity risk assessment of Prothioconazole + Spiroxamine EC 460, it is the opinion of the notifier that chronic risk assessments for combined exposure to fish and invertebrates do not need to be presented. Formulations break down into their respective components very shortly after they enter the environment therefore an assessment of the risk to any toxic effects of Prothioconazole + Spiroxamine EC 460 is only applicable immediately after exposure. The chronic risk assessment considers the potential risks over much longer time periods, by which point the formulation will have broken down into the two individual active substances which will then behave independently. Thus, the chronic risk of spiroxamine and prothioconazole are considered to be covered by the risk assessments for the individual active substances. However, an assessment of the contribution that each active substance makes to the overall chronic mixture toxicity has been presented below.

Careful selection of the active substance endpoints is necessary for use in the predicted mixture toxicity calculations so that accurate predictions of toxicity can be made as well as meaningful comparisons with the measured formulation toxicity data. The table below presents the relevant endpoints for spiroxamine and prothioconazole which have been used in the mixture toxicity calculations along with a discussion of the suitability of these values. Note that the endpoints for each active substance have been selected in an attempt to use the same test species for spiroxamine, prothioconazole and Prothioconazole + Spiroxamine EC 460. As a result, the lowest endpoint for each active has not always been used in order to ensure a meaningful comparison between predicted and measured toxicity can be made.

Table CP 10.2-31 Individual active substance endpoints used for the mixture toxicity calculations for Prothioconazole + Spiroxamine EC 460

Organism group	Spiroxamine (µg a.s./L)	Prothioconazole (µg a.s./L)	Comments
Fish	*O. mykiss*: 96-hr LC_{50} 18,500	*O. mykiss*: 96-hr LC_{50} 1,830	Endpoints have been taken from studies using the same test species and equivalent test designs. Both LC_{50} values are bound and are therefore considered to be an accurate reflection of the actual toxicity. Thus, the endpoints are considered suitable for use in a mixture toxicity calculation.
	L. macrochirus: 96-hr LC_{50} 4,130	*L. macrochirus*: 96-hr LC_{50} 4,590	Endpoints have been taken from studies using the same test species and equivalent test designs. Both LC_{50} values are bound and are therefore considered to be an accurate reflection of the actual toxicity. Thus, the endpoints are considered suitable for use in a mixture toxicity calculation.
	O. mykiss: ELS NOEC 14.2	*O. mykiss*: ELS NOEC 308	Endpoints have been taken from studies using the same test species and equivalent test designs therefore the endpoints are considered suitable for use in a mixture toxicity calculation.
Aquatic invertebrate	*D. magna*: 48-hr EC_{50} 3,000	*D. magna*: 48-hr EC_{50} 1,300	Endpoints have been taken from studies using the same test species and equivalent test

Organism group	Spiroxamine (µg a.s./L)	Prothioconazole (µg a.s./L)	Comments
			designs. Both EC_{50} values are bound and are therefore considered to be an accurate reflection of the actual toxicity. Thus, the endpoints are considered suitable for use in a mixture toxicity calculation.
	D. magna: 21-day NOEC: 34	*D. magna*: 21-day NOEC 560	Endpoints have been taken from studies using the same test species and equivalent test designs therefore the endpoints are considered suitable for use in a mixture toxicity calculation.
Algae	*P. subcapitata*: 120-hr E_rC_{50} 19.43	*P. subcapitata*: 72-hr E_rC_{50} 2,180	It is noted that a potentially lower endpoint is available for spiroxamine using this species. However, with an unbound value of >8.14 µg a.s./L it was not considered reliable for mixture toxicity assessment as the actual E_rC_{50} could have been much higher. Endpoints have been taken from studies using the same test species and equivalent test designs. Both EC_{50} values are bound and are therefore considered to be an accurate reflection of the actual toxicity. Both values have used the growth rate endpoint and are therefore comparable. Thus, the endpoints are considered suitable for use in a mixture toxicity calculation.

<u>Formulation composition</u>

Prothioconazole + Spiroxamine EC 460 has the following nominal composition:

Spiroxamine: 300 g/L (30.5% w/w using a density of 0.984 g/mL)

Prothioconazole: 160 g/L (16.3% w/w using a density of 0.984 g/mL)

Thus, the total active substance content was 460 g/L (46.8% total active substance) therefore the relative proportions of each active substance were as follows:

Spiroxamine: 65.2% or 0.652

Prothioconazole: 34.8% or 0.348

The maximum initial PEC_{sw} values of spiroxamine and prothioconazole can also be compared in order to determine the relative composition of the mixture in surface waters. The FOCUS Step 2 NEU and SEU PEC_{sw} values for prothioconazole for applications of 1.25 L/ha to Spring and Winter cereals are both 2.048 µg a.s./L. The FOCUS Step 2 NEU and SEU PEC_{sw} values for spiroxamine for applications of 1.25 L/ha to Spring and Winter cereals are 3.673 and 5.194 µg a.s./L, respectively. Taking the highest PECsw value for spiroxamine of 5.194 µg a.s./L gives the following relative proportions:

Spiroxamine: 71.7% or 0.717

Prothioconazole: 28.3% or 0.283

The relative proportions of spiroxamine and prothioconazole in the nominal composition of Prothioconazole + Spiroxamine EC 460 when compared with the relative proportions of spiroxamine and prothioconazole in the maximum initial Step 2 PEC_{sw} values are within 10% of each other. For the

mixture toxicity calculations the relative proportions from the nominal formulation composition have therefore been used.

Predicted mixture toxicity

Using the active substance endpoints from the table above the calculated mix-CA values in terms of total active substance and formulation have been determined and presented below.

Table CP 10.2-32 Calculated mixture toxicity in terms of total a.s. content for Prothioconazole + Spiroxamine EC 460

Organism group / species		Endpoint	p[1]/ LC_{50} or EC_{50}	p[2]/ LC_{50} or EC_{50}	Calculated Mix-CA (µg a.s./L)[3]	Calculated Mix-CA (µg form./L)[4]
Fish	*O. mykiss*	LC_{50}	0.0000353	0.000190	4,438	9,483
	L. macrochirus	LC_{50}	0.0000915	0.0000758	5,979	12,776
	O. mykiss	NOEC	0.0459	0.00113	21.3	45.4
Aquatic invert	*D. magna*	EC_{50}	0.000217	0.000268	2,062	4,406
	D. magna	NOEC	0.0192	0.000621	50.6	108
Algae	*P. subcapitata*	EC_{50}	0.0336	0.000160	29.7	63.4

[1] proportion of spiroxamine (p_s = 0.652)
[2] proportion of prothioconazole (p_a = 0.348)
[3] $EC_{50mix\text{-}CA} = 1 / (p_s/EC_{50}^1 + p^2/EC_{50}^2)$
[4] Based on a total a.s. purity of 46.8% w/w assuming a Spiroxamine content of 300 g/L, Prothioconazole content of 160 g/L and a formulation density of 0.984 g/mL
Calculated values have been rounded for presentation purposes

For acute fish the predicted LC_{50} of the formulation is 9.48 and 12.8 mg product/L for rainbow trout and bluegill sunfish, respectively. For acute aquatic invertebrates the predicted EC_{50} of the formulation is 4.41 mg product/L for *Daphnia magna*. For algae the predicted E_rC_{50} of the formulation is 0.0634 mg product/L for *Pseudokirchneriella subcapitata*.

Toxic units

The toxic unit (TU) of a mixture is defined as the sum of the TU of each individual substance in the mixture therefore the predicted data can also be examined for the contribution of the two separate active substances to the mixture toxic units.

If the toxicity of the mixture is largely explained by the toxicity of a single a.s., a sufficient protection level might be achieved by simply basing the risk assessment on the toxicity data for that single 'driver'. Hence, where CA provides a reliable estimate of the toxicity of the given mixture (EC_{Xppp}) and the largest part of the sum of toxic units (*i.e.* ≥ 90 %) calculated for the measured mixture toxicity (EC_{Xppp}) by Equation 14 comes from a single a.s., it can be concluded that this component drives the overall mixture toxicity.

Equation 14:

$$\sum_{i=1}^{n} TU_i = \sum_{i=1}^{n} \frac{c_i}{ECx_i}$$

Where;
TU_i: Toxic units of component *i*
c_i: concentration of a mixture component *i*
ECx_i: concentration of component *i* provoking x% effect

The calculated toxic units for spiroxamine and prothioconazole for each organism group are presented in the table below along with the percentage contribution of each active to the overall toxicity of the mixture.

Table CP 10.2-33 Toxic unit calculations and contribution of each active substance to the predicted toxicity of Prothioconazole + Spiroxamine EC 460

Organism group	Active substance	C_i/ECx$_i$	TU_i	ΣTU	Contribution (%)
Fish – acute (*O. mykiss*)	Spiroxamine	30.5 / 18500	0.00165	0.0106	15.6
	Prothioconazole	16.3 / 1830	0.00891		84.4
Fish – acute (*L. macrochirus*)	Spiroxamine	30.5 / 7130	0.00428	0.00783	54.6
	Prothioconazole	16.3 / 4590	0.00355		45.4
Fish - chronic	Spiroxamine	30.5 / 14.2	2.15	2.20	**97.6**
	Prothioconazole	16.3 / 308	0.0529		2.40
Aquatic invertebrate - acute	Spiroxamine	30.5 / 3000	0.0102	0.0227	44.8
	Prothioconazole	16.3 / 1300	0.0125		55.2
Aquatic invertebrate - chronic	Spiroxamine	30.5 / 34	0.897	0.926	**96.9**
	Prothioconazole	16.3 / 560	0.0291		3.14
Algae	Spiroxamine	30.5 / 19.43	1.57	1.58	**99.5**
	Prothioconazole	16.3 / 2180	0.00748		0.474

% contribution values in **bold** are >90% thereby suggesting that spiroxamine is the main driver of toxicity

For the acute fish data using both species it is concluded that neither active substance alone is driving the acute toxicity to fish. The same situation exists for the acute invertebrate data with neither active substance driving the acute toxicity to aquatic invertebrates.

However, for the chronic fish, chronic aquatic invertebrate and the algal data it is very clear that the contribution spiroxamine makes to the toxicity of the mixture is well over 90%. Thus, it can be concluded that spiroxamine is the driver of the toxicity, and hence the risk assessment, for chronic fish, chronic aquatic invertebrate and algae.

Although a chronic mixture toxicity calculation has not been performed, the chronic risk assessment for fish and aquatic invertebrates with spiroxamine on its own would be considered sufficient to cover the chronic risk assessment for the mixture.

Model Deviation Ratio (MDR)

The calculated endpoints have been compared against the available experimentally determined endpoints for the formulation to give a model deviation ratio (MDR) using the following equation:

MDR = Mix-CA / experimental formulation endpoint

Calculated MDR values are presented below.

Table CP 10.2-34 Model Deviation Ratio values from comparison of the predicted toxicity to the measured toxicity of Prothioconazole + Spiroxamine EC 460

Organism group	Endpoint	Calculated Mix-CA (µg form./L)	Measured formulation toxicity (µg form./L)	MDR	Comment
Acute fish	LC_{50}	9,483	*O. mykiss* 96-hr LC_{50}: 6,570	1.44	MDR values within the range 0.2 - 5 therefore there is agreement with the principles of concentration addition (CA)
Acute aquatic invertebrate	EC_{50}	4,406	*D. magna* 48-hr EC_{50}: 6,300	0.699	
Algae	E_rC_{50}	63.4	*P. subcapitata* 72-hr E_rC_{50}: 147	0.431	

For acute fish, acute aquatic invertebrates and algae the MDR values are well within the range of 0.2 - 5.0. Thus, the results demonstrate that the combined effects of spiroxamine and prothioconazole are in agreement with the principles of concentration addition. In accordance with the Aquatic Guidance Document, where there is good agreement between the observed (*i.e.* experimentally determined) and calculated toxicities, the experimentally measured mixture toxicity values should be used in the risk assessment.

Thus, the formulation specific risk assessment presented above for Prothioconazole + Spiroxamine EC 460 using acute fish, acute invertebrate and algal data covers the risk assessment for the combined effects of the two active substances. A chronic mixture risk assessment for fish and invertebrates has not been presented but it has already been demonstrated that the spiroxamine component of the formulation is driving the chronic risk to fish and invertebrates (toxic units >90%) therefore no specific chronic mixture calculations are considered to be necessary.

Refined risk assessment for the chronic risk to fish from exposure to spiroxamine

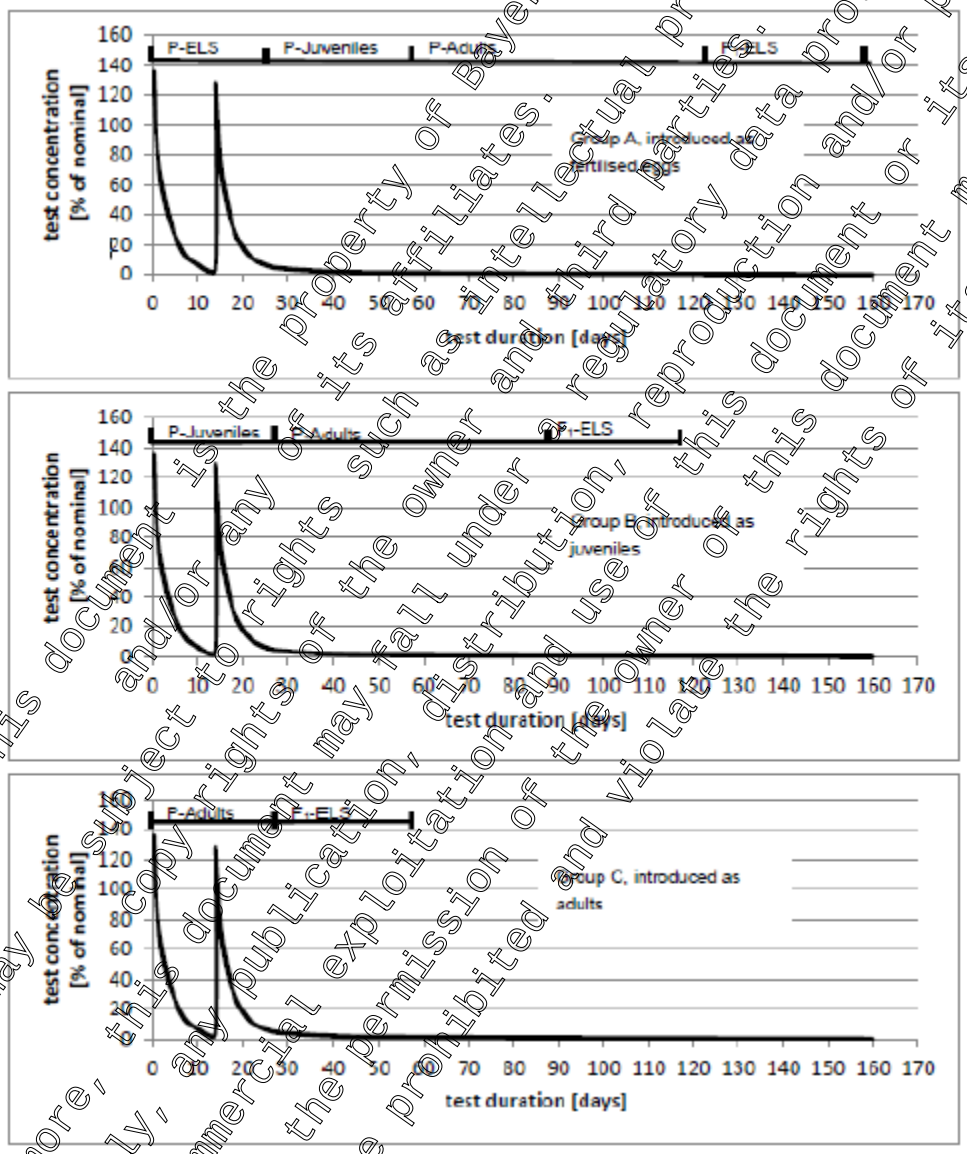
Two Fish Full Life Cycle (FFLC) studies are available using spiroxamine technical. The first study (M-304458-02-1) was conducted under continuous exposure conditions (*i.e.* a flow-through test design) and provided a NOEC of 2.6 µg a.s./L. In the Assessment report for spiroxamine provided by the Rapporteur Member State (RMS) Germany in September 2009 (Volume 3, Annex B9, pp. 963-969), the RMS calculated an EC_{10} of 2 µg a.s./L. The RMS considered the EC_{10} to be an adequate endpoint for regulation, *i.e.* for Tier 1 risk assessment. This EC_{10} value has been recalculated as part of the current Renewal of Approval of spiroxamine, following an assessment of the statistical methods (M-760413-01-1), and a value of 1.88 µg a.s./L has been determined. Thus, the EC_{10} of 1.88 µg a.s./L has been used in the Tier I chronic fish risk assessment presented above.

The second FFLC study (M-467979-03-1) was conducted as a refinement study and simulated a peak-exposure scenario in the presence of sediment. This refined FFLC study provided NOEC and EC_{10} values of 15.8 and 23.3 µg a.s./L, respectively. In line with the EFSA Aquatic Guidance Document (EFSA PPR Panel 2013), refined exposure laboratory toxicity tests can be used in the higher tier risk assessment (Tier 2C), if the exposure regime of the higher-tier effect study covers the predicted exposure regime in edge-of-field water bodies.

The objective of the refined FFLC study was to assess the effects of spiroxamine peak-exposure on different life stages of zebrafish (*Danio rerio*) under static conditions in a water-sediment system. A full summary of the study has been provided in Document M-CA Section 8 and further details can be found in the study report (M-467979-03-1). In short, zebrafish were exposed to two successive pulses of spiroxamine separated by a 14-day interval, during a full life-cycle that included F0 early life stages, juvenile growth, adult reproduction, and early life stages of the F1 generation (Figure 10.2-01). The two

spiroxamine pulses therefore expose the fish at several different sensitive development stages (fertilised eggs, newly hatched larvae, 4-week old juveniles during growth, and adult during reproduction). The target nominal peak-exposure concentrations were 12, 24, 48 and 192 µg a.s./L. Mean measured peak-exposure concentrations of spiroxamine were 15.3, 30.8, 68.0 and 265.7 µg a.s./L for the first pulse, and 16.2, 30.0, 59.7 and 244 µg a.s./L for the second pulse. The overall measured test concentrations were determined by taking a mean of the two initial peak exposure concentrations.

Figure CP 10.2-01 Spiroxamine peak exposure of different life stages of zebrafish. Exposure started with fertilised eggs, juveniles and adults in Groups A, B and C respectively. (Source M-467979-03-1)



P: Parental generation; F1: Filial 1 generation; ELS: Early Life Stage

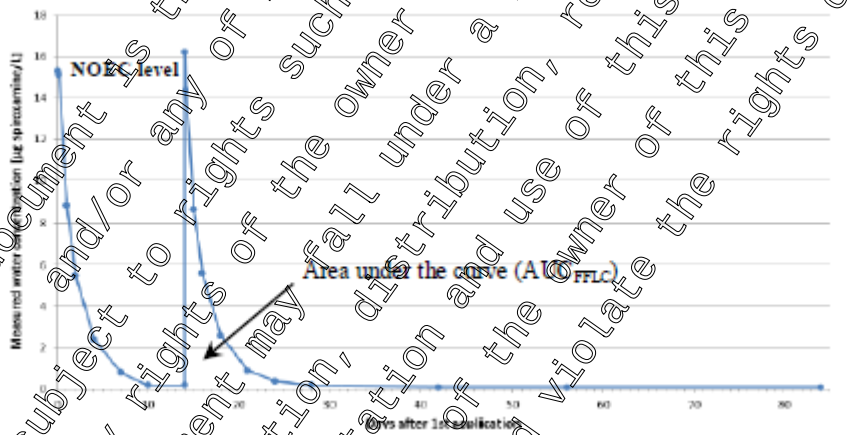
The most sensitive biological endpoint was survival of the F1-larvae of group C (parental generation exposed as adults). The corresponding NOEC was determined to be 15.8 µg a.s./L and the EC_{10} was determined to be 23.3 µg a.s./L (expressed as mean measured concentrations). The first and second pulses corresponding to the peak concentrations of 15.3 and 16.2 µg a.s./L, respectively, resulted in no mortality in the exposed fish. The NOEC value of 15.8 µg a.s./L was therefore calculated as the mean of the exposure concentrations at the first and second peaks.

Both EC_{10} and NOEC values have been calculated for the most sensitive endpoint of the refined-exposure FFLC study as outlined in the EFSA Aquatic Guidance Document (2013) and in data requirements (Comission Regulation 283/2013). EFSA Supporting publication 2015: EN-924 states that where a reliable median EC_{10} could be calculated, then the lower between this value and the NOEC should be used. Looking at the study, it is clear that with a limited number of tested doses (4 plus controls) the study has been designed to derive a NOEC and not an EC_x. However, the dose intervals cover an adequate range for EC_x calculation with effect values of 4.4 %, 10.9 %, 50.9 %, and 94.4 % for 15.8, 30.4, 63.9, and 255 µg/L, respectively. Because the confidence limits span a broad range and taking into account the effects actually measured in the test, the EC_{10} was considered as less meaningful, and the NOEC of 15.8 µg/L was deemed more appropriate for risk assessment. Overall, the NOEC is found more adequate to be used for the risk assessment based on the refined-exposure FFLC study.

The comparison of the two fish full life cycle studies with spiroxamine conducted under continuous and peak exposure conditions, respectively, showed in both studies the same sensitive endpoints indicating consistency of the results of both studies (*i.e.* effects on survival of F1-generation, survival of F0-generation, sex ratio of adults, weight and vitellogenin biomarker for adult females).

Questions have previously been raised regarding this study and the possibility that not all developmental life stages of the fish have been sufficiently exposed in light of the exposure profile achieved in the test. Figure 10.2-02 presents the measured concentrations of spiroxamine determined in the study.

Figure CP 10.2-02 Measured spiroxamine concentration in water samples from study M-467979-03-1, following application of 12 µg a.s./L (NOEC, nominal concentration) on Day 0 and Day 14



Mean measured peak concentrations of Spiroxamine were 15.3 µg a.s./L and 16.2 µg a.s./L for the first and second pulses, respectively. Mean measured NOEC was therefore 15.8 µg a.s./L

The study comprised three test groups in which different life stages of the fish were used at the start of the exposure test. Thus, fertilised eggs, newly hatched larvae, 4-week old juveniles during growth, and adults during reproduction would have been exposed to one or both of the exposure pulses. In a refined laboratory exposure test, such as this, the fundamental concept is to use a more representative exposure regime which mimics the situation in the field. This is achieved by using an exposure regime which is considered to be realistic to worst case when compared to the relevant FOCUS profile(s). A direct consequence of taking this modified exposure approach is that the exposure will not be 'worst case' for the duration of the exposure period. The only way that this can be achieved is under the continuous renewal conditions used in the standard Tier I test design. Thus, by deliberate design, a modified exposure test like this cannot expose every possible part of the organism's life for the duration of the test. However, what it can do is provide results which are considered to be more realistic based on what is likely to occur in the field and therefore the results determined from the test are more realistic and, hence, more relevant. The refined FFLC study has included all of the critical life stages of a fish from embryo through to adult therefore all life stages are considered to have been covered by the test design. Whilst the maximum exposure may not have coincided with, for example, the day of hatching, this

critical phase has still been covered by the exposure regime and hatching embryos would have been exposed to spiroxamine. It is therefore considered that the test design was appropriate to sufficiently expose the fish at all sensitive life stages but under conditions which are much more realistic in relation to the environment following application of spiroxamine. Whilst it is accepted that the concentrations of spiroxamine reduced to below LOQ 10 days after application, ultimately this is the purpose of the study as it recreates the typical exposure that would occur in the field.

The results of the refined FFLC study can be compared to the results of the other available Tier I chronic fish data. Two flow through fish early life stage (ELS) studies with rainbow trout using continuous flow-through conditions are available in which the test concentrations were maintained from embryo addition through to juvenile fish. In the first study (M-006232-01-1) a NOEC was not established but a 93-day EC_0 of 14 µg a.s./L was derived as a surrogate. In the second ELS study (M-006449-02-1) a 96-day NOEC of 14.2 µg a.s./L was derived. In the standard FFLC study with zebrafish, which also used continuous flow-through test conditions, a 230-day NOEC value of 2.6 µg a.s./L was achieved. Although these three studies have used two different fish species and have used different test durations, the results are considered to be largely consistent with each other and provide a good reference point for the chronic NOEC for fish following constant exposure to spiroxamine. The NOEC achieved in the refined FFLC study of 15.8 µg a.s./L is therefore considered to be at a remarkably similar level to those values already achieved. Indeed, the difference between the two FFLC studies using the same test species, but different exposure regimes, is only a factor of 6. Thus, the results achieved under the modified exposure test conditions are highly similar to those results achieved under constant exposure in which all sensitive life stages were exposed to worst case conditions. This would strongly suggest that the exposure regime of the refined FFLC study was sufficient for the toxic effects of spiroxamine to manifest themselves. It is therefore considered that the zebrafish in the refined FFLC study were adequately exposed to spiroxamine, including the most sensitive developmental stages.

The EFSA Aquatic Guidance Document stipulates certain conditions under which modified chronic exposure studies can be used to derive a Chronic RAC for use in a refined risk assessment. These are:

- The (repeated pulsed) exposure regime in the refined laboratory toxicity test is realistic to worst case when compared with the relevant predicted (modelled) field exposure profile.
- The duration of the test is long enough to allow the observation of delayed effects
- The refined chronic RAC is compared with the $PEC_{sw;max}$

In order for the refined Tier 2C RAC value of 1.58 µg a.s./L to be used in the risk assessment it is necessary to compare the exposure profile achieved in this study with the exposure profiles for each of the relevant FOCUS scenarios that did not pass the risk assessment using the Tier I RAC of 0.188 µg a.s./L. Only those FOCUS scenarios that are considered to be covered by the refinement study, in terms of the exposure profile, can use the Tier 2C RAC value in the refined risk assessment. The Tier 2C RAC value would then be compared to the $PEC_{sw;max}$ as required by the Aquatic Guidance Document.

A full analysis of each relevant FOCUS exposure profile in relation to the exposure in the refined FFLC study, along with an assessment of the applicability for use in a refined risk assessment, will be conducted and submitted as part of the top-up submission. Consideration over the length of the test in relation to assessing delayed effects will also be provided.

Illustrative refined risk assessment

The table below presents an illustrative refined risk assessment, using the Tier 2C RAC value of 1.58 µg a.s./L for the scenarios that did not demonstrate an acceptable risk at Tier I. Note that this has been presented purely in order to demonstrate the potential that the Tier 2C RAC has to refine the risk assessment and to demonstrate an acceptable chronic risk to fish. The current proposed mitigation has been maintained here but it should also be noted that, for scenarios where the Tier 2C RAC can be used, a lower level of mitigation could possibly be used.

Table CP 10.2-35 Summary of potential refined risk assessment for the proposed uses of Prothioconazole + Spiroxamine EC 460 on cereals using the Tier 2C RAC of 1.58 µg a.s./L

Proposed use of Prothioconazole + Spiroxamine EC 460 to cereals (L/ha)		FOCUS scenarios for which further refinement is required based on the Tier I RAC of 0.188 µg a.s./L	Step 4 PEC_{sw} using a 30 m nsbz with a 20 m vfs (µg a.s./L)	PEC/RAC ratio based on Tier 2C RAC of 1.58 µg a.s./L
1 x 1.25 Spring cereals	Early	D1 Ditch	0.192	0.122
	Late	D1 Ditch	0.192	0.122
		R4 Stream	0.224	0.142
1 x 1.25 Winter cereals	Early	D2 Ditch	0.192	0.122
	Late	D1 Ditch	0.192	0.122
		D2 Ditch	0.192	0.122
		D6 Ditch	0.192	0.122
2 x 1.25 Spring cereals	Early	D1 Ditch	0.236	0.149
		R4 Stream	0.372	0.235
	Late	D1 Ditch	0.219	0.139
		R4 Stream	0.227	0.144
2 x 1.25 Winter cereals	Early	D2 Ditch	0.192	0.122
		R1 Stream	0.239	0.151
		R3 Stream	0.237	0.150
		R4 Stream	0.397	0.251
	Late	D1 Ditch	0.239	0.151
		D2 Ditch	0.241	0.153
		D6 Ditch	0.192	0.122
		R1 Stream	0.241	0.153

PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; nsbz: no spray buffer zone; vfs: vegetated filter strip

It is clear that in situations where the Tier 2C RAC value is considered to be suitable for use in the risk assessment (*i.e.* the refined FFLC study exposure is realistic to worst case in relation to that particular FOCUS scenario exposure profile), all relevant scenarios for all of the proposed uses of Prothioconazole + Spiroxamine EC 460 could result in PEC/RAC ratios <1, thereby allowing for the demonstration of an acceptable chronic risk to fish when mitigation in the form of a 30 m no-spray buffer zone with a 20 m vegetated filter strip is applied.

Secondary poisoning risk assessment

Spiroxamine

The Log P_{ow} of spiroxamine is 2.79 and 2.98 at pH 7 for diastomers A and B, respectively but at pH 9 these values are 4.88 and 5.08, respectively therefore a specific risk assessment to address the potential risks of accumulation and biomagnification in the aquatic food chain is required. The worst case BCF value has been determined to be 87 L/kg (M-006479-01-1). With a Log P_{ow} of >3 there is the potential for accumulation of spiroxamine within the aquatic food chain, *via* secondary poisoning of birds and mammals, following consumption of contaminated fish.

In accordance with the EFSA Aquatic Guidance Document[7] and the EFSA Bird & Mammal Guidance Document[8], a secondary poisoning risk assessment has been conducted in order to assess the potential risks of transfer of lipophilic compounds, such as spiroxamine, through the food chain.

The biomagnification factor (BMF) is defined as the relative concentration in a predatory animal compared with the concentration in its prey (BMF = $C_{predator}/C_{prey}$). The Regulatory Acceptable Concentration for secondary poisoning (RAC_{sp}) is calculated for both birds and mammals using the following equations:

$$\mathbf{RAC_{sp}} = \frac{\mathbf{NOAEL_{bird}}}{\mathbf{5 \times 0.159 \times BCF_{fish} \times BMF}} \quad \textbf{or} \quad \frac{\mathbf{NOAEL_{mammal}}}{\mathbf{5 \times 0.142 \times BCF_{fish} \times BMF}}$$

In accordance with the Aquatic Guidance Document, for the Tier I secondary poisoning risk assessment a default BMF value of 1 is used for compounds with a BCF < 2,000 L/kg. The values of 0.159 and 0.142 are multiplication factors based on a 1000 g bird eating 159 g fish per day and a 3000 g mammal eating 425 g of fish per day. The worst case BCF value of 87 L/kg has also been used in the calculations. The NOAEL for birds is 5.40 mg a.s./kg bw/day and the NOAEL for mammals is 21.0 mg a.s./kg bw/day.

The following RAC_{sp} values have been calculated:

RAC_{sp} bird = 0.0781 mg/L = **78.1 µg a.s./L**

RAC_{sp} mammal = 0.340 mg/L = **340 µg a.s./L**

According to the Aquatic Guidance Document:

If RAC_{sp} > 21-day TWA PEC_{sw} - Acceptable risks; no further action necessary

If RAC_{sp} < 21-day TWA PEC_{sw} - Refinement is necessary

The highest FOCUS Step 3 TWA PEC_{sw} value for the uses in cereals has been determined to be 2.029 µg a.s./L (D1 ditch, Spring cereals 2 x 375 g a.s./ha, early application). This value has therefore been used in the risk assessment. It is clear that the RAC_{sp} values for birds and mammals (78.1 and 340 µg a.s./L, respectively) are greater than the worst case FOCUS Step 3 TWA PEC_{sw} value for spiroxamine (2.029 µg a.s./L) following the representative uses. Thus, the concentrations of spiroxamine will not accumulate within the tissues of birds and mammals at concentrations high enough to cause possible harmful effects following consumption of contaminated fish. A low risk from bioaccumulation within the aquatic food chain is therefore concluded.

Prothioconazole

Discussion of the specific endpoints for prothioconazole are not considered part of the Renewal of Approval for spiroxamine therefore a specific secondary poisoning risk assessment for prothioconazole has not been conducted here. However, in the EFSA Conclusion for prothioconazole (EFSA Scientific Report (2007) 106, 1-98) it is stated that *"the risk to earthworm- and fish-eating birds and mammals from secondary poisoning was considered to be low based on the low BCF in fish and short depuration rates for both prothioconazole and the desthio metabolite"*. A low risk from bioaccumulation within the aquatic food chain is therefore concluded.

Summary of aquatic risk assessment

[7] Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters. EFSA Journal 2013; 11 (7): 3290

[8] European Food Safety Authority; Guidance Document on Risk Assessment for Birds & Mammals on request from EFSA. EFSA Journal 2009; 7(12): 1438. Doi: 10.2903/j.efsa.2009.1438

For spiroxamine exposure, the acute risks to fish, acute and chronic risks to aquatic invertebrates, risks to aquatic macrophytes and to sediment dwelling organisms was demonstrated to be acceptable following all proposed uses of Prothioconazole + Spiroxamine EC 460 without the need for any mitigation measures. For the chronic risks to fish, risks to algae and to those organisms covered by the mesocosm study (algae and invertebrates), application mitigation was required.

For all organism groups, with the exception of the chronic risk to fish from spiroxamine exposure, acceptable risks could be demonstrated for all relevant FOCUS scenarios for all proposed uses of Prothioconazole + Spiroxamine EC 460 when a 20 m no-spray buffer zone with a 20 m vegetated filter strip is applied as a mitigation measure. For the chronic risk to fish from spiroxamine exposure a **30 m no-spray buffer zone with a 20 m vegetated filter strip** was necessary in order to demonstrate an acceptable chronic risk for many of the relevant FOCUS scenarios. However, not all scenarios passed the chronic fish risk assessment, as detailed below.

Proposed use of Prothioconazole + Spiroxamine EC 460 to cereals (L/ha)		**FOCUS scenarios for which acceptable risks have been demonstrated using a 20 m nsbz with a 20 m vfs**	**FOCUS scenarios for which further refinement is required**
1 x 1.25 Spring cereals	Early	D1 Stream, D3 Ditch, D4 Pond*, D4 Stream, D5 Pond*, D5 Stream, R4 Stream	D1 Ditch
	Late	D1 Stream, D3 Ditch, D4 Pond*, D4 Stream, D5 Pond*, D5 Stream	D1 Ditch, R4 Stream
1 x 1.25 Winter cereals	Early	D1 Ditch, D1 Stream, D2 Stream, D3 Ditch, D4 Pond*, D4 Stream, D5 Pond*, D5 Stream, D6 Ditch, R1 Pond*, R1 Stream, R3 Stream, R4 Stream	D2 Ditch
	Late	D1 Stream, D2 Stream, D3 Ditch, D4 Pond*, D4 Stream, D5 Pond*, D5 Stream, R1 Pond*, R1 Stream, R3 Stream, R4 Stream	D1 Ditch, D2 Ditch, D6 Ditch
2 x 1.25 Spring cereals	Early	D1 Stream, D3 Ditch, D4 Pond*, D4 Stream, D5 Pond*, D5 Stream,	D1 Ditch, R4 Stream
	Late	D1 Stream, D3 Ditch, D4 Pond*, D4 Stream, D5 Pond*, D5 Stream,	D1 Ditch, R4 Stream
2 x 1.25 Winter cereals	Early	D1 Ditch, D1 Stream, D2 Stream, D3 Ditch, D4 Pond*, D4 Stream, D5 Pond*, D5 Stream, D6 Ditch, R1 Pond*	D2 Ditch, R1 Stream, R3 Stream, R4 Stream
	Late	D1 Stream, D2 Stream, D3 Ditch, D4 Pond*, D4 Stream, D5 Pond*, D5 Stream, R1 Pond, R3 Stream, R4 Stream	D1 Ditch, D2 Ditch, D6 Ditch, R1 Stream,

nsbz: no spray buffer zone; vfs: vegetated filter strip
* Scenario for this use passes the risk assessment at Step 3 therefore no mitigation required

For all proposed uses of Prothioconazole + Spiroxamine EC 460 at least one full FOCUS scenario passed the chronic fish risk assessment for spiroxamine exposure. However, refinement of the chronic fish risk assessment is necessary for some of the individual scenarios. Justification to use a refined chronic fish RAC value to further refine the spiroxamine risk assessment will be provided in the top-up submission.

Following a formulation specific risk assessment, considering spray drift only, the risks to aquatic organisms were demonstrated to be acceptable when a 5 m no-spray buffer zone is used as application

mitigation. This mitigation is covered by the mitigation of a **30 m no-spray buffer zone with a 20 m vegetated filter strip** already proposed above.

The risks to aquatic organisms from exposure to the metabolites KWG 4168-desethyl (M01), KWG 4168-despropyl (M02) and KWG 4169-N-oxide (M03) was demonstrated to be acceptable following all proposed uses of Prothioconazole + Spiroxamine EC 460. For KWG 4168-acid (M06) acceptable risks to aquatic organisms have been demonstrated for the proposed uses of Prothioconazole + Spiroxamine EC 460 on Spring and Winter cereals (early and late applications) at 1 x 1.25 L/ha. However, for the proposed uses at 2 x 1.25 L/ha, possible risks to aquatic organisms have been identified following exposure to M06. Further environmental fate data for this metabolite are currently being generated and the PEC_{sw} modelling will be updated and submitted as part of the top-up submission.

No unacceptable risks to aquatic organisms from exposure to prothioconazole and the metabolites prothioconazole-S-methyl and 1,2,4-Triazole, following applications of Prothioconazole + Spiroxamine EC 460 to cereals at 1.25 L/ha, were demonstrated. The chronic risks to fish from exposure to prothioconazole-desthio were demonstrated to be acceptable when application in the form of a 10 m no-spray buffer zone with run-off reduction in the form of a vegetated filter strip was applied. It is noted that this mitigation is covered by the mitigation proposed based on the spiroxamine risk assessments.

The potential risks from bioaccumulation of spiroxamine within the aquatic food chain have been demonstrated to be low.

Biodiversity

No relevant scientifically peer-reviewed open literature could be found on spiroxamine or its major metabolites, from an ecotoxicological perspective, on aquatic organisms. Therefore, it is considered that the potential impact of the active substance on biodiversity and the ecosystem, including potential indirect effects *via* alteration of the food web, are covered by the risk assessment for aquatic organisms in this section and in the ED hazard assessment.

Sumaries of the available data with Prothioconazole + Spiroxamine EC 460 are presented below.

CP 10.2.1 Acute toxicity to fish, aquatic invertebrates, or effects on aquatic algae and macrophytes

Data Point:	KCP 10.2.1/01
Report Author:	[redacted]
Report Year:	2002
Report Title:	Acute toxicity of JAU 6476 & Spiroxamine EC 460 to fish (Oncorhynchus mykiss)
Report No:	DOM 21059
Document No:	M-039959-01-1
Guideline(s) followed in study:	U.S.-EPA-540/9-85-006 (1982/1985) OPPTS 850.1075 (pulic draft, 1996) EU Directive 92/69/EEC, C.1 (1992) OECD no. 203 (rev. 1992) U.S.-EPA-FIRA § 72-1 Canadian PMRA Ref.: DACO 9.5.2.1 EU Council Directive 91/414/EEC (1991)
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and accepted RAR (2010)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

In a 96-hour acute toxicity study, rainbow trout (*Oncorhynchus mykiss*) were exposed to JAU 6476 & Spiroxamine EC 460 at nominal concentrations of 1.25, 2.50, 5.00, 10.0 and 20.0 mg test item/L under static conditions.

Sub-lethal effects were observed in the groups exposed to 5.00 mg test item/L. These included: lying inactive on the bottom of the aquarium, turning dark in colouration, loss of equilibrium with lateral deviation from normal orientation, displaying mucous excretions from the intestine and lying on their sides and backs.

The 96-hour LC_{50} was 6.57 mg test item/L, and the NOEC based on sub-lethal effects was 2.5 mg test item/L.

I. Materials and Methods

Materials

Test Material JAU 6476 & Spiroxamine EC 460

Lot/Batch #: 06920/0045 (0019)

Purity: JAU 6476: 160 g/L (16.3%), spiroxamine: 296 g/L (30.1%)

Description: Yellow liquid

Stability of test compound: t 1/2 > 500h (pH 4-9, 50°C); t 1/2 > 1 year (pH 7,9 / 25°C)

Reanalysis/Expiry date: 02 November 2001

Density: Not reported

Treatments

Test rates:

Nominal:	1.25, 2.50, 5.00, 10.0, and 20.0 mg test item/L
Initial measured:	0.200, 0.398, 0.826, 1.68, and 2.58 mg a.s./L

Solvent/vehicle:	Not reported
Analysis of test concentrations:	Yes, initial mean measured concentrations between 79 to 103% of nominal
Test organisms	
Species:	Rainbow trout, *Oncorhynchus mykiss*, mean body weight: 2.4 g, mean body length: 6.1 cm
Source:	Dr. Rosengarten, D-49124 Oesede-Georgsmarienhütte, FRG
Acclimatisation period:	14 days
Feeding:	Commercial trout food during acclimatisation period, not fed for 48 h prior to or during the study
Treatment for disease:	Not necessary
Test design	
Test vessel:	40 L glass aquaria of 32 x 36 x 38 cm
Test medium:	Reconstituted water, prepared by adding salt stock solutions to demineralised water
Replication:	One vessel per treatment
No. animals/vessel:	10 fish per test concentration
Duration of test:	96 hours
Environmental test conditions	
Temperature:	10.9 – 11.5 °C
Dissolved oxygen:	98 – 100% of the air saturation value
pH:	7.0 – 7.4
Photoperiod:	16 hours light : 8 hours dark

Study Design

This study was conducted in order to assess the acute toxicity of JAU 6476 & Spiroxamine EC 460 to rainbow trout (*Oncorhynchus mykiss*) in a static test over 96 hours.

The fish used in the study had a mean wet weight of 2.4 ± 0.4 g and a mean body total length of 6.1 ± 0.4 cm. Biomass loading of fish in the test aquaria was 0.60 g fish/L test medium.

Aquaria were glass and measured 32 x 36 x 38 cm , with a test volume of 40 L. To each aquaria was added 10 fish per test concentration.

Nominal test concentrations were 1.25, 2.50, 5.00, 10.0, and 20.0 mg test item/L, equivalent to 0.204, 0.408, 0.815, 1.63, and 3.26 mg a.s./L based on JAU 6476. Relevant concentrations of the test item were added to the test media directly. Initial mean measured concentrations were 0.200, 0.398, 0.826, 1.68, and 2.58 mg a.s./L.

Dissolved oxygen ranged from 98 to 100% of the air saturation value in all aquaria. The pH of test solutions ranged from 7.0 to 7.4 over the course of the test, and the temperature ranged from 10.9 to 11.5 °C.

Test organisms were not fed for 48 hours before or during the study. Feed analysed for wanted contaminants only.

LC_{50} values and 95%-confidence intervals were calculated every 24 hours using a computer program, which estimated LC_{50} using one of three statistical techniques: moving average, binominal probability or probit analysis (appropriate method determined according to data characteristics).

Analytical method

Samples of water were analysed using the validated analytical method 00586, report reference M-012801-01-1 (**see Doc MCP Section 5**).

Results and Discussion

Validity criteria were not assessed in the study report.

At Day 0 measured concentrations ranged between 0.200 and 2.58 mg a.s./L and the percent of nominal ranged between 79 and 103%. At Day 2 measured concentrations ranged between 0.149 and 1.20 mg a.s./L. At Day 4 measured concentrations ranged between 0.114 and 0.501 mg a.s./L. Initial mean measured concentrations were 0.200, 0.398, 0.826, 1.68, and 2.58 mg a.s./L.

All results have been expressed in terms of nominal test concentrations.

Table CP 10.2.1/01-1 Nominal and measured concentrations of JAU 6476 during the study

Nominal concentration (mg test item (*a.s.*)/L)	Mean measured concentration in mg a.s./L			Percent of nominal (day 0)
	Day 0	Day 2	Day 4	
Control	<0.02	<0.02	<0.02	--
1.25 (*0.204*)	0.200	0.149	0.114	98
2.50 (*0.408*)	0.398	0.299	0.228	98
5.00 (*0.815*)	0.826	0.652	0.501	101
10.0 (*1.63*)	1.68	1.20[1]		103
20.0 (*3.26*)	2.58	-	-	79

[1] Water samples of 10.0 mg test item/L (nominal) were analysed on day 1 due to 100 percent mortality in this level
- no observations, all fish dead

No mortalities or observable symptoms of intoxication were observed in the control, 1.25 or 2.50 mg test item/L nominal concentration groups. All fish in the 5.00 mg test item/L test group at 24 hours exposure and in the 10.0 mg test item/L at 4 hours exposure were observed with either toxic symptoms or mortality. All the fish in the 20.0 mg test item/L test group died after 4 hours of exposure.

Table CP 10.2.1/01-2 Cumulative mortality and behavioural observations

Nominal concentration (mg test item/L)	4 hour		24 hour		48 hour		72 hour		96 hour	
	Dead	Obs.	Dead	Obs.	Dead	Obs.	Dead	Obs.	Dead	Obs.
Control	0	0	0	0	0	0	0	0	0	0
1.25	0	0	0	0	0	0	0	0	0	0

Nominal concentration (mg test item/L)	4 hour		24 hour		48 hour		72 hour		96 hour	
	Dead	Obs.	Dead	Obs.	Dead	Obs.	Dead	Obs.	Dead	Obs.
2.50	0	0	0	0	0	0	0	0	0	0
5.00	0	0	0	10 TS, DF, SD, BO	1	10 TS, DF, SD, BO, SR	1	10 BO, DF, SD	1	10 TS, DF, SD, BO
10.0	0	10 TS, DF	10	10	-	-	-	-	-	-
20.0	10	10	-	-	-	-	-	-	-	-

Dead fish are added to the sum of fish with symptoms
BO: laid inactive on the bottom of the aquarium
DF: turned dark in colouration
SR: laid on their sides or backs
TS: showed loss of equilibrium with lateral deviation from their normal orientation
SD: displayed mucous excretions from the intestine
-: no observations, all fish dead

Table CP 10.2.1/01-3 Calculated LC_{50} values

Exposure period (hour)	LC_{50} (mg test item/L)	95% C.I (mg test item/L)
24	7.07	5.00-10.0
48	6.57	5.00 - 10.0
72	6.57	5.00 - 10.0
96	6.57	5.00 - 10.0

Table CP 10.2.1/01-4 Summary of endpoints after 96-hour exposure to JAU 6476 & Spiroxamine EC 460

Endpoint	NOEC	LOEC	LC_{50}
mg test item/L	2.50	5.00	6.57

Conclusion

After 96 hours exposure to JAU 6476 & Spiroxamine EC 460, the acute LC_{50} of *Oncorhynchus mykiss* was 6.57 mg test item/L (equivalent to 1.98 mg spiroxamine/L and 1.07 mg prothioconazole/L) with 95% confidence intervals of 5.00 to 10.0 mg test item/L. The 96-hour NOEC and LOEC were 2.50 and 5.00 mg test item/L, respectively.

Assessment and conclusion by applicant:

The study was conducted to the original 1992 version of the OECD 203 test guideline. Validity criteria according to the current OECD 203 (2019) guideline have been assessed and were met:

- Control mortality must not exceed 10% at the end of the test (actual: 0%)
- Dissolved oxygen concentration in all test vessels to be ≥60% of the air saturation value (actual: 98 to 100%)

- Analytical measurement of test concentrations is compulsory (analysis was performed)

The study is therefore considered acceptable.

The acute LC_{50} of *Oncorhynchus mykiss* was 6.57 mg test item/L (equivalent to 1.98 mg spiroxamine/L and 1.07 mg prothioconazole/L).

Data Point:	KCP 10.2.1/02
Report Author:	[redacted]; [redacted]
Report Year:	2002
Report Title:	Acute toxicity of JAU 6476 EC 160 & spiroxamine 300 to water fleas (Daphnia magna)
Report No:	DOM 22017
Document No:	M-069578-01-1
Guideline(s) followed in study:	U.S.-EPA-FIFRA § 72-2 Canadian PMRA Ref.: DACO 9.3.2 EU Council Directive 91/414/EEC (1991) OPPTS 850.1010 (public draft, 1996) (modified) EU Directive 92/69/EEC, C.2 (1992) OECD no. 202 (rev. 1984, draft 2000)
Deviations from current test guideline:	Daphnids were 10/vessel instead of the recommended 5/vessel Three replicate vessels used, 4 required by current guidance
Previous evaluation:	yes, evaluated and accepted RAR (2010)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The 48-hour acute toxicity of JAU 6476 EC 160 & Spiroxamine 300 to *Daphnia magna* was studied under static conditions. Test species were exposed to nominal test concentrations of 0.56, 1.0, 1.8, 3.2, 5.6, 10 and 18 mg formulation/L for 48 hours. Immobilisation and sub-lethal effects were observed after 24 hours and 48 hours.

The 48-hour EC_{50} was 6.3 mg formulation/L. The 48-hour NOEC based on immobilisation was 3.2 mg formulation/L.

I. Materials and Methods

Materials

Test Material JAU 6476 EC 160 & Spiroxamine 300

Lot/Batch #: 06920/0045(0019)

Purity: JAU 6476: 15.8%; Spiroxamine 300: 30.3%

Description: Clear dark yellow liquid

Stability of test compound: Not reported

Reanalysis/Expiry date: 30 April 2002

Density: 0.981 g/mL

Treatments

Test rates:	Nominal: control, 0.56, 1.0, 1.8, 3.2, 5.6, 10 and 18 mg formulation/L Measured: <0.0091, 0.071, 0.138, 0.237, 0.448, 0.824, 1.41, 2.60 mg JAU 6476/L at test start
Solvent/vehicle:	None
Analysis of test concentrations:	Yes, measured concentration of JAU 6476 were 81 to 94 % of nominal on day 0 and 16 to 89 % of nominal on day 2

Test organisms

Species:	*Daphnia magna,* first instar (< 24 hrs old)
Source:	In-house culture, originally from Bundesgesundheitsamt, Berlin, Germany
Acclimatisation period:	None reported, however culture was raised in the laboratory for over 10 years
Feeding:	Not fed during the test
Treatment for disease:	None reported

Test design

Test vessel:	100 mL glass beakers containing 50 mL test solution, covered with plexi glass plates
Test medium:	M7-medium
Replication:	Three replicates
No. animals/vessel:	10 daphnids per vessel
Duration of test:	48 hours

Environmental test conditions

Temperature:	18 – 22°C
Dissolved oxygen:	Test start: 8.1 – 8.9 mg/L (approx. 87.99 – 96.68% saturation) Test end: 8.2 – 8.8 mg/L (approx. 89.80 – 96.37% saturation)
pH:	Test start: 7.9 – 8.0 Test end: 7.9 – 8.0
Photoperiod:	16 hrs light: 8 hrs dark

Study Design

This study was conducted in order to assess the acute toxicity of JAU 6476 EC 160 & Spiroxamine 300 to the water flea *Daphnia magna* over 48 hours.

First instar *Daphnia magna* were used in the test from an in-house culture, aged <24 hours. First instar daphnids were separated from older daphnids by careful mesh screening.

Test vessels were 100 mL beakers containing 50 mL test solution, covered with a plexi glass plate. Beakers were held in a climatic chamber for 48 hours between 18 and 22 °C (±1) under a 16 hours light to 8 hours dark photoperiod.

Nominal concentrations of the test substance were prepared by dilution of stock solutions. Stock solutions were stirred using a magnetic stirrer for 10 minutes. Nominal concentrations were 0.56, 1.0, 1.8, 3.2, 5.6, 10 and 18 mg formulation/L.

To each test concentration were added ten first instar *Daphnia magna* using a pipette. Three replicates were used per concentration.

After 24 and 48 hours, water fleas were assessed visually for survivors, *i.e.* animals with swimming movements within 15 seconds of gentle agitation of the test vessel. Additional observations for sub-lethal effects were also made.

Temperature, oxygen content and pH of the test water was determined using electronic measuring equipment. Temperature was measured at the start and end of the study in one vessel of the control and one vessel of the highest test concentration. Oxygen content and pH were determined both at test initiation and test termination.

The EC_{50} values and the 95 percent confidence limits were manually determined using probit analysis after the maximum-likelihood method.

Analytical method

Samples of water were analysed using the validated analytical method 00586, report reference M-012801-01-1 (**see Doc MCP Section 5**).

II. Results and Discussion

The study was deemed valid in the report as control mortalities were less than 10%.

Test concentrations were determined at test start in all test concentrations. Mean measured concentrations of JAU 6476 were 87.7% of nominal at test start and were 50.8% of nominal at test end. As Day 0 concentrations were achieved, the results are based on nominal concentrations.

Table CP 10.2.1/02-1 Analysed test concentrations of JAU 6476 in test solutions

Nominal concentrations		**Day 0**		**Day 2**	
(mg formulation/L)	**(mg JAU 6476/L)**	**Analysed conc. (mg JAU 6476/L)**	**Percent of nominal**	**Analysed conc. (mg JAU 6476/L)**	**Percent of nominal**
Control	---	< 0.0091*)	---	< 0.0091*)	---
0.56	0.088	0.071	81	0.014	16
1.0	0.16	0.138	86	0.042	26
1.8	0.28	0.237	85	0.117	42
3.2	0.51	0.448	88	0.239	47
5.6	0.88	0.824	94	0.564	64
10	1.6	1.41	88	1.15	72
18	2.8	2.60	93	2.48	89
		Average: 87.7		Average: 50.8	

*) = lowest standard concentration used during analysis

After 24 hours exposure to JAU 6476 EC 160 & Spiroxamine 300, cumulative immobility of *Daphnia magna* was 0, 0, 0, 0, 0, 0, 3 and 43% in the control and 0.56, 1.0, 1.8, 3.2, 5.6, 10 and 18 mg formulation/L, respectively.

Table CP 10.2.1/02-2 Water flea toxicity of JAU 6476 EC 160 & Spiroxamine 300 (24 hours)

Nominal concentration (mg formulation/L)	Replicate No.	Mobile Daphnids	Immobilised Daphnids (%)*	Mobile Daphnids showing symptoms	Mobile Daphnids showing symptoms (%)*
Control	1 2 3	10 10 10	0	-	0
0.56	1 2 3	10 10 10	0	-	0
1.0	1 2 3	10 10 10	0	-	0
1.8	1 2 3	10 10 10	0	-	0
3.2	1 2 3	10 10 10	0	-	0
5.6	1 2 3	10 10 10	0	-	0
10	1 2 3	10 10 9	3 ± 6	-	0
18	1 2 3	5 6 6	43 ± 6	[3][6.4] [1][6.4]	13 ± 15

[ ] number of living animals showing symptoms at observed
* given as mean ± standard deviation (n–1 method)
Symptoms:
1) Quick, trembling antennae movements.
2) Frequency of antennae movements clearly increased.
3) Frequency of antennae movements clearly decreased.
4) Hardly any movements perceivable.
5) Swimming movements showed coordination disturbances.
6) Animals lie at the bottom.
7) Animals cling to the water surface.
8) Animals cling together in clusters.

After 48 hours exposure to JAU 6476 EC 160 & Spiroxamine 300, cumulative immobility of *Daphnia magna* was 0, 0, 0, 0, 0, 50, 83 and 100% in the control and 0.56, 1.0, 1.8, 3.2, 5.6, 10 and 18 mg formulation/L, respectively.

Table CP 10.2.1/02-3 Water flea toxicity of JAU 6476 EC 160 & Spiroxamine 300 (48 hours)

Nominal concentration (mg formulation/L)	Replicate No.	Mobile Daphnids	Immobilised Daphnids (%)*	Mobile Daphnids showing symptoms	Mobile Daphnids showing symptoms (%)*
Control	1 2 3	10 10 10	0	-	0
0.56	1 2 3	10 10 10	0	-	0
1.0	1 2 3	10 10 10	0	-	0
1.8	1 2 3	10 10 10	0	-	0
3.2	1 2 3	10 10 10	0	-	0
5.6	1 2 3	5 4 6	50 ± 10	[2] 6.4 [2] 6.4 [1] 6.4	17 ± 6
10	1 2 3	5 0 2	83 ± 15	-	0
18	1 2 3	0 0 0	100	-	13 ± 15

[ ] number of living animals showing symptoms at observed
* given as mean ± standard deviation (n-1 method)
Symptoms:
1) Quick, trembling antennae movements.
2) Frequency of antennae movements clearly increased.
3) Frequency of antennae movements clearly decreased.
4) Hardly any movements perceivable.
5) Swimming movements showed coordination disturbances.
6) Animals lie at the bottom.
7) Animals cling to the water surface.
8) Animals cling together in clusters.

The endpoints determined by statistical analysis were as follows.

Table CP 10.2.1/02-4 Summary of endpoints after 48-hr exposure to JAU 6476 EC 160 & Spiroxamine EC 300

Endpoint (mg formulation/L)	EC_{50}	NOEC	LOEC
24-hours	19	10	18
48-hours	6.3	3.2	5.6

III. Conclusion

After 48 hours exposure to JAU 6476 EC 160 & Spiroxamine EC 300, the 48-hour EC_{50} to *Daphnia magna* was 6.3 mg formulation/L (equivalent to 1.91 mg spiroxamine/L and 0.995 mg prothioconazole/L, respectively), with 95% confidence intervals of 2.0 to 19 mg formulation/L. The 48-hour NOEC and LOEC were 3.2 and 5.6 mg/formulation/L, respectively.

Assessment and conclusion by applicant:

The study was conducted to an older version of the OECD 202 test guideline. The study has therefore been assessed against the most recent version (April 2004).

Validity criteria according to OECD 202 (2004) were met:

- Mortality/immobilisation in the control to not exceed 10% (actual: 0.0%)
- Dissolved oxygen concentration at test termination to be ≥3 mg/L in all test vessels (actual: 8.2 to 8.9 mg/L)

This study used three replicates of 10 organisms which is a deviation from current guideline requirements of four replicates of 5 organisms but as the total number of organisms used in this study was greater than that required, this deviation is not considered to have had a detrimental impact and the results are still considered to be valid.

The study is therefore considered to be acceptable.

The 48-hour EC_{50} to *Daphnia magna* was 6.3 mg formulation/L (equivalent to 1.91 mg spiroxamine/L and 0.995 mg prothioconazole/L, respectively).

Data Point:	KCP 10.2.1/03
Report Author:	[redacted]
Report Year:	2002
Report Title:	Toxicity of JAU 6476 & KWG 4168 EC 460 to Pseudokirchneriella subcapitata (formerly Selenastrum capricornutum) in a 72-hour algal growth inhibition test
Report No:	841378
Document No:	M-077013-02-1
Guideline(s) followed in study:	OECD no. 201 (1984) EU Commission Directive 92/69/EEC, C.3 (1992)
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and accepted RAR (2010)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

In a 72-hour toxicity study, cultures of *Pseudokirchneriella subcapitata* were exposed under static conditions to JAU 6476 160 EC & KWG 4168 300 at nominal test concentrations of 0.010, 0.032, 0.10, 0.32, 1.0, and 3.2 mg/L.

The test item had a statistically significant inhibitory effect on the growth (biomass and growth rate) of *Pseudokirchneriella subcapitata* after an exposure period of 72 hours at the lowest test concentration of the formulation of nominal 0.010 mg/L and above.

The growth inhibition in the treated algal culture as compared to the control ranged from 3.7% to 69.7%. The biomass inhibition in the treated algal culture compared to the control ranged from 5.6% to 87.0%.

The E_rC_{50} and E_bC_{50} values were determined to be 0.16 and 0.015 mg/L, respectively.

I. Materials and Methods

Materials

Test Material JAU 6476 160 EC & KWG 4168 300

Lot/Batch #:	06920/0045(0019)
Purity:	JAU 6476: 16.1% w/w (158.42 g/L) KWG 4168: 29.8% w/w (293.23 g/L)
Description:	Clear red-brown
Stability of test compound:	JAU 6476: Stable to hydrolysis KWG 4168: Stable to hydrolysis and photodegradation
Reanalysis/Expiry date:	7 May 2003
Density:	0.984 g/mL

Treatments

Test rates:	Nominal: 0.010, 0.032, 0.10, 0.32, 1.0, and 3.2 mg/L
Solvent/vehicle:	None
Analysis of test concentrations:	Yes, mean measured concentrations of JAU 6476 and JAU 6476-desthio were 83 – 118% of nominal

Test organisms

Species:	*Pseudokirchneriella subcapitata*, (formerly *Selenastrum capricornutum*)
Source:	SAG, Universitat Gottingen, D-37073 Gottingen, Germany

Test design

Test vessel:	50-mL Erlenmeyer flasks with glass covers
Test medium:	Sterile, deionised water reconstituted to OECD 201 media
Replication:	6 replicates for the control, 3 per test concentration
Initial cell density:	1 x 10^4 cells/mL
Duration of test:	72 hours

Environmental test conditions

Temperature:	23 °C
pH:	Test start: 7.8 – 7.9 Test end: 7.7 – 8.2
Photoperiod:	24-hour a day at 8650 – 9470 lux

Study Design

The green alga *Pseudokirchneriella subcapitata* was exposed to JAU 6476 160 EC & KWG 4168 300 over 72 hours to determine inhibition of the growth. The test concentrations were based on the results of a non-GLP range-finding test.

The test was started by inoculation of 1 x 10^4 algal cells per mL test medium. These cells were taken from an exponentially growing pre-culture, which was set up 3 days prior to the test under the same conditions as in the test. There were three replicates per test concentration and six replicates of the control. Volumes of 15 mL algal suspension for each replicate were continuously stirred by magnetic stirrers in 50 mL Erlenmeyer flasks covered with glass dishes. They were incubated in a temperature controlled water bath at a temperature of 23 °C and continuously illuminated at a measured light intensity of about 9130 lux. The pH of the test solution ranged from 7.8 to 7.9 at the start of the test, and at the end ranged from 7.7 to 8.2.

The concentrations of JAU 6476 160 EC & KWG 4168 300 tested were 0.010, 0.032, 0.10, 0.32, 1.0, and 3.2 mg/L. The reference substance potassium dichromate was tested once per year to demonstrate satisfactory test conditions.

Small volumes of the test media and the control (1.0 mL) were taken out of all test flasks after 24, 48 and 72 hours exposure, and were not replaced. The algal cell densities in the samples were determined by counting with an electronic particle counter (Coulter Counter®, Model ZM), with at least two measurements per sample. In addition, after 72 hours exposure, a sample was taken from the control and from a test concentration with reduced algal growth (nominal 0.032 mg/L). The shape of the algal cells was microscopically examined in these samples.

The algal cell densities in the samples were determined by counting with an electronic particle counter, with at least two measurements per sample.

For the quantification of the concentrations of the formulation JAU 6476 160 EC & KWG 4168 300, the concentrations of the active ingredient JAU 6476 and of its degradation product JAU 6476-desthio in the test media were analytically determined at 0 and 72 hours.

Analytical method

Samples of water were analysed using the validated analytical method 00586, report reference M-012801-01-1 (**see Doc MCP Section 5**).

Samples of water were analysed using the validated analytical method 00684, report reference M-079449-01-1 (**see Doc MCP Section 5**).

II. Results and Discussion

Validity criteria according to the study report were met:

- The biomass in the control cultures should have increased exponentially by a factor of ≥16 within the 72-hour test period. This corresponds to a specific growth rate of 0.92 per day (actual: 77 times increase)

Mean measured concentrations of JAU 6476 and JAU 6476-desthio in the freshly prepared test media were 74 to 118% of nominal values. Mean measured concentrations over the duration of the test ranged between 83 and 118% of nominal. All results have been presented in terms of the nominal test concentrations.

Table CP 10.2.1/03-1 Mean measured concentrations of JAU 6476 and JAU 6476-desthio during the test

Nominal formulation concentration (mg/L)	Nominal JAU 6476 concentration (µg/L)	Mean measured concentration of total JAU 6476 and JAU 6476-desthio	
		µg/L	% of nominal
0.010	16	14.5	90
0.032	51	42.6	83
0.10	160	189	118
0.32	512	535	104
1.0	1600	1790	112
3.2	5120	4410	86

The test item had a statistically significant inhibitory effect on the growth (biomass and growth rate) of *Pseudokirchneriella subcapitata* after the exposure period of 72 hours at the lowest test concentration of the formulation of 0.010 mg/L and above.

The growth inhibition in the treated algal culture as compared to the control ranged from 3.7% to 69.7%. The biomass inhibition in the treated algal culture compared to the control ranged from 5.6% to 87.0%.

Table CP 10.2.1/03-2 Percent inhibition of growth rate and biomass (relative to control) in *Pseudokirchneriella subcapitata* exposed to JAU 6476 160 EC & KWG 4168 300

Nominal concentration (mg/L)	% inhibition in growth rate			% inhibition in biomass		
	24 h	48 h	72 h	24 h	48 h	72 h
Control	-	-	-	-	-	-
0.010	10.3	9.6*	19.1*	17.5	22.9*	44.7*
0.032	7.4	16.7*	27.7*	12.2	31.9*	58.3*
0.10	12.4	31.6*	53.3*	20.5	50.8*	79.8*
0.32	7.5	47.4*	68.3*	12.2	60.0*	87.0*

Nominal concentration (mg/L)	% inhibition in growth rate			% inhibition in biomass		
	24 h	48 h	72 h	24 h	48 h	72 h
1.0	3.8	48.5*	68.6*	5.6	58.5*	86.9*
3.2	3.7	42.5*	69.7*	6.8	55.3*	85.8*
* Statistically different from the control (Dunnett's test, one-sided, α=0.05)						

The microscopic examination of the algal cells after 72 hours exposure showed no difference between algae growing in test media containing the test item at a nominal concentration of 0.032 mg/L and the algal cells in the controls. There were no obvious effects on the shape and size of the algal cells growing in test media containing the test item at up to and including this nominal concentration.

A summary of the relevant endpoints determined in the report are presented below:

Table CP 10.2.1/03-3 Summary of derived endpoints

Growth rate	
E_rC_{50}:	0.16 mg/L (confidence limits of 0.04 to 0.60 mg/L)
E_rC_{10}:	<0.010 mg/L
E_rC_{90}:	>0.32 mg/L
LOE_rC:	≤0.010 mg/L
NOE_rC:	<0.010 mg/L
Biomass	
E_bC_{50}:	0.015 mg/L (confidence limits of 0.005 to 0.028 mg/L)
E_bC_{10}:	<0.010 mg/L
E_bC_{90}:	>0.32 mg/L
LOE_bC:	≤0.010 mg/L
NOE_bC:	<0.010 mg/L

III. Conclusion

Exposure to JAU 6476 160 EC & KWG 4168 300 over 72 hours caused statistically significant inhibition of the growth (biomass and growth rate) of the green alga *Pseudokirchneriella subcapitata* at concentrations of ≥0.01 mg/L.

The E_rC_{50} was determined to be 0.16 mg/L (equivalent to 0.0477 mg spiroxamine/L and 0.0258 mg prothioconazole/L, respectively), with 95% confidence limits of 0.04 to 0.60 mg/L.

The E_bC_{50} was determined to be 0.015 mg/L (equivalent to 0.00447 mg spiroxamine/L and 0.00242 mg prothioconazole/L, respectively), with 95% confidence limits of 0.005 to 0.028 mg/L.

Assessment and conclusion by applicant:

The study was conducted to the OECD test guideline 201 (1984), the current version of which is the OECD 201 "Alga, Growth Inhibition Test", adopted March 2006 and corrected July 2011. Validity criteria have therefore been re-assessed according to the current 2011 version of the guideline and have been met:

- The biomass in the control cultures should have increased exponentially by a factor of ≥16 within the 72-hour test period. This corresponds to a specific growth rate of 0.92 per day (actual: 77).
- The mean coefficient of variation for section-by-section specific growth rates (days 0-1, 1-2 and 2-3) in the control cultures <35% (actual: 11.5%).
- The coefficient of variation of average specific growth rates during the whole test period in replicate control cultures <7% (actual: 2.64%)

The study is considered to be valid and acceptable.

The E_rC_{50} was determined to be 0.16 mg/L (equivalent to 0.0477 mg spiroxamine/L and 0.0258 mg prothioconazole/L, respectively).

The data have been subjected to statistical re-evaluation and the results have been presented in the following study summary.

Data Point:	KCP 10.2.1/23
Report Author:	[redacted]
Report Year:	2020
Report Title:	Calculation of EC10, EC20 and EC50 values for Pseudokirchneriella subcapitata with JAU 6476 160 EC & KWG 4168 300 in an algal growth inhibition test
Report No:	0471836-ECO29
Document No:	M-761433-01-1
Guideline(s) followed in study:	None
Deviations from current test guideline:	None
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	No, not conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The report M-077013-02-1 on the effects of exposure to JAU 6476 160 EC & KWG 4168 300 on the growth of algae (*Pseudokirchneriella subcapitata*) did not provide estimates of EC_{10} or EC_{20} values. Therefore, these values have been calculated alongside the E_rC_{50} in accordance with the Annex to Com. Reg. 283/2013.

The resulting EC_{50} value for yield at 72 h was 8.46 µg/L. EC_{10} and EC_{20} values could not be determined. For growth rate after 72 h, the EC_{20} and EC_{50} values were 6.34 and 147.43 µg/L, respectively. An EC_{10} value could not be determined.

I. Methods

The statistical evaluation was performed with statistical software ToxRatPro v3.3.0.

Effect concentrations with 10, 20 and 50% effect from the test item treatment when compared to the control were determined for yield and growth rate after 72 hours exposure. A linear Probit analysis was performed on both the yield and growth rate data to determine EC_x values. Confidence limits were estimated according to Fieller's theorem.

II. Results and Discussion

Yield at 72 hours

Regarding the calculation of the EC_{50} value for yield at 72 h, a statistically significant concentration/response was found ($p(F)^1<0.001$) for this parameter.

The resulting EC_{50} value and the respective confidence intervals are represented in the following table below:

Table CP 10.2.1/23-1 Results of the Probit analysis of yield at 72 h: Selected effective concentrations (EC_x) of the test item and their 95%-confidence limits

Parameter	Yield		
	EC_{10} (95 % confidence interval) [µg/L]	EC_{20} (95 % confidence interval) [µg/L]	EC_{50} (95 % confidence interval) [µg/L]
Effect on yield at 72 h	n.d.	n.d.	8.46 (3.34 – 13.59)

n.d.: not determined since value is beyond the tested concentrations

The resulting EC_{50} value of 8.46 (95%CL: 3.34 – 13.59) µg/L met the goodness of fit criteria, showing a significant concentration/response relationship and therefore the estimated EC_{50} values considered reliable. EC_{10} and EC_{20} values could not be calculated.

Growth rate at 72 hours

Regarding the calculation of EC_{20} and EC_{50} values for yield at 72 h, a statistically significant concentration/response was found ($p(F) < 0.001$) for this parameter.

The resulting EC_{20} and EC_{50} values and the respective confidence intervals are represented in the following table below.

Table CP 10.2.1/23-2 Results of the Probit analysis of growth rate at 72 h: Selected effective concentrations (EC_x) of the test item and their 95%-confidence limits

Parameter	Growth rate		
	EC_{10} (95 % confidence interval) [µg/L]	EC_{20} (95 % confidence interval) [µg/L]	EC_{50} (95 % confidence interval) [µg/L]
Effect on growth rate at 72 h	n.d.	6.34 (1.46 – 25.07)	147.43 (85.11 – 250.35)

n.d.: not determined since value is beyond the tested concentrations

The resulting EC_{20} and EC_{50} values of 6.34 (95%CL: 1.46 – 25.07) and 147.43 (95%CL: 85.11 – 250.35) µg/L, respectively, meet the goodness of fit criteria showing a significant concentration/response relationship and therefore the estimated EC_x values are considered reliable. An EC_{10} could not be calculated.

III. Conclusion

The resulting EC_{50} value for yield at 72-hours was determined to be 8.46 µg/L. EC_{10} and EC_{20} values for yield could not be calculated. The EC_{20} and EC_{50} values for growth rate at 72-hours were determined to be 6.34 and 147.43 µg/L. An EC_{10} for growth rate could not be calculated.

Assessment and conclusion by applicant:

The statistical re-evaluation of the data has determined reliable EC_{50} values for yield and reliable EC_{20} and EC_{50} values for growth rate. Reliable EC_{10} and EC_{20} values for yield and an EC_{10} for growth rate could not be calculated.

The E_rC_{50} determined in this re-evaluation work of 147 µg/L is slightly lower than the E_rC_{50} determined in the original study report of 160 µg/L therefore the E_rC_{50} of 147 µg/L shall be taken as the critical endpoint determined from this study.

The values determined in the re-evaluation work are considered to be fully valid.

Data Point:	KCP 10.2.1/04
Report Author:	[redacted]
Report Year:	2002
Report Title:	Toxicity of JAU 6476 & KWG 4168 EC 460 to the aquatic higher plant Lemna gibba (duckweed) in a 7-day semistatic growth inhibition test
Report No:	841376
Document No:	M-077038-01-1
Guideline(s) followed in study:	OECD no. 221 (draft october 2000)
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and accepted RAR (2010)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

This study was conducted to determine toxic effects of JAU 6476 160 EC & KWG 4168 300 on the growth of the freshwater aquatic plant *Lemna gibba*. In a 7-day growth inhibition study, *Lemna gibba* (duckweed) were exposed to JAU 6476 160 EC & KWG 4168 300 at nominal concentrations of 2.0, 6.4, 20, 64, 200 and 640 µg/L under semi-static conditions in accordance with the OECD 221 guideline.

The 7-day NOEC was determined to be 6.4 µg/L. The 7-day EC_{50} values based on growth rate, area under growth curves (AUC) and final biomass were 57, 39 and 67 µg/L, respectively.

There were no compound-related phytotoxic effects.

I. Materials and Methods

Materials

Test Material JAU 6476 160 EC & KWG 4168 300

Lot/Batch #: 06920/0045(0019)

Purity: JAU 6476: 16.1% w/w (158.42 g/L)
KWG 4168: 29.8% w/w (293.23 g/L)

Description: Clear red-brown liquid

Stability of test compounds: Stable to hydrolysis; stable to hydrolysis and degradation

Reanalysis/Expiry date: JAU 6476: May 2003; JAU 6476-desthio: Jan 2003

Density: 0.984 g/mL

Treatments

Test rates: Nominal: 2.0, 6.4, 20, 64, 200 and 640 µg/L

Solvent/vehicle:	None
Analysis of test concentrations:	Yes, mean measured concentrations 71 – 90% of the nominal values (exception of the lowest test concentration at 223% of nominal.)
Test organisms	
Species:	Duckweed (*Lemna gibba)*
Source:	Syngenta AG, Ecological Science, CH-4002 Basel, Switzerland
Acclimatisation period:	4 weeks
Test design	
Test vessel:	250 mL glass dishes (8.5 cm diameter) filled with 150 mL test medium (water depth approx.. 25 mm)
Test medium:	20X AAP medium
Replication:	Three test vessels
No. animals/vessel:	Three colonies (with four fronds each) per vessel (12 fronds/vessel)
Duration of test:	7 days
Environmental test conditions	
Temperature:	23°C
pH:	7.4-8.8
Photoperiod:	Continuous illumination of 7130 Lux by fluorescent tubes (Philips TLD 36W/840)

Study Design

Duckweed was exposed for 7 days under semi-static conditions to nominal concentrations of the formulation; 2.0, 6.4, 20, 64, 200 and 640 µg/L. Test vessels were incubated in a temperature-controlled water bath at 23 ˚C and continuously illuminated at 7130 lux by fluorescent tubes for the duration of the study. Three colonies, consisting of four fronds each, were transferred from the pre-culture into the test vessels in a randomised order. Additionally, a test vessel containing test medium of the lowest test concentration and a test vessel containing test medium of the highest test concentration were incubated under test conditions without *Lemna* plants during the first test medium renewal interval.

The test design included three replicates per test concentration and control. Each replicate consisted of a 250-mL-glass dish (diameter of 8.5cm) filled with 150mL test medium resulting in a water depth of approximately 25 mm. The test vessels were covered with glass lidsTest media was renewed on Day 3 and Day 5.

The colonies were inspected on days 3, 5 and 7 for changes in frond number, colony number and appearance (discolouration, sinking root length or other abnormalities). Fronds visibly projecting over the edge of the mother frond were counted as separate fronds. The dry weight of a sample of fronds was determined and at test termination, the dry weight of all colonies per test vessel was determined.

For the quantification of the concentrations of the formulation JAU 6476 160 EC & KWG 4168 300, the concentrations of the active ingredient JAU 6476 and of its degradation product JAU 6476-desthio in the test media were analytically determined. Quadruplicate samples (4 x 20 ml) were taken from the stock solutions and from the freshly prepared test media of all test concentrations and the control at the start of the test and at the test medium preparations on Day 3 and 5. For the determination of the maintenance of the concentrations of the active ingredient JAU 6476 during the test medium renewal

periods, quadruplicate samples (4 x 20 ml) of all test concentrations and the control were taken after the test medium renewal periods on Day 3 and 5, and at the end of the test. The aged test media were sampled by pouring the test media from the test vessels into the sampling flasks.

The 7-day EC_{10}. EC_{50} and EC_{90} and their 95% confidence limits were calculated by Probit analysis for the growth parameters of growth rate (r), AUC and final biomass. The 7-day NOEC and LOEC for *Lemna* growth following exposure were determined by testing growth parameters, at the different test concentrations, on statistically significant differences to the control values by multiple Dunnett-tests.

Analytical method

Samples of water were analysed using the validated analytical method 00586, report reference M-012801-01-1 (**see Doc MCP Section 5**).

Samples of water were analysed using the validated analytical method 00684, report reference M-079449-01-1 (**see Doc MCP Section 5**).

II. Results and Discussion

Validity criteria according to the OECD221 test guideline, to which the study was conducted, were met.

- Doubling time of frond number in the control to be less than 2.5 days (60 h), corresponding to an average specific growth rate of 0.275/d (actual: 2.0 days)

In the samples of the freshly prepared stock solutions, the total concentration of the active substance JAU 6476 and JAU 6476-desthio was determined to be in the range of 101 to 114% of the nominal value demonstrating the correct preparation of the stock solutions (Table A4 to A6). The total concentrations of JAU 6476 and JAU 6476-desthio found in the freshly prepared test media were between 50 to 270% of the nominal values. Higher concentrations were found in most of the aged samples from the end of the test medium renewal periods. These differences are due to the difficult analysis of JAU 6476 and JAU 6476-desthio at the lower test levels. The mean measured concentrations (calculated as the average over all measurements per test concentration) ranged between 71 and 90% of the nominal values with the exception of the lowest test concentration with 223% of nominal.

All results have been presented in terms of the nominal test concentrations.

Table CP 10.2.1/04-1 Mean measured concentrations of JAU 6476 and JAU 6476-desthio during the test

Nominal formulation concentration (mg/L)	Nominal JAU 6476 concentration (µg/L)	Mean measured concentration of total JAU 6476 and JAU 6476-desthio	
		µg/L	% of nominal
2.0	0.32	0.71	223
6.4	1.02	0.82	81
20	3.2	2.6	83
64	10.2	7.2	71
200	32	25.7	80
640	102	92.1	90

At the test concentrations of the formulation of nominal 2.0 and 6.4 ug/L, the average growth of *Lemna gibba* on Day 7 was not statistically significantly lower that in the control (results of Dunnett-tests, one-sided, $\alpha = 0.05$). The growth parameters of average specific growth rate (r), AUC, and the mean dry weight of the plants after the test period of 7 days were statistically significantly reduced at the test concentration of 20 µg/L and at all higher test concentrations. At the highest test concentration of 640

µg/L, the growth of *Lemna gibba* was nearly completely inhibited. At the test concentrations of 20 µg/L and above, the colonies resolved into small colonies or single fronds. Single fronds without roots or with shortened roots were observed. At the test concentrations of nominal 200 and 640 µg/L, discoloured fronds were observed.

Table CP 10.2.1/04-2 Total number of fronds and colonies per test vessel at the counting dates

Nominal concentration of formulation (µg/L)	Vessel no.	Frond number (#F) and colony number (#C) per test vessel							
		0 hr (day 0)		72 hr (day 3)		120 hr (day 5)		168 hr (day 7)	
		#F	#C	#F	#C	#F	#C	#F	#C
Control	1	12	3	29	3	64	6	121	12
	2	12	3	36	3	66	6	134	12
	3	12	3	29	3	59	6	123	12
	Mean	12.0	3.0	31.3	3.0	63.0	6.0	132.7	12.0
	SD	0.0	0.0	4.0	0.0	3.6	0.0	8.2	0.0
2.0	1	12	3	32	3	74	6	143	13
	2	12	3	33	3	65	6	139	12
	3	12	3	30	3	60	5	126	13
	Mean	12.0	3.0	31.7	3.0	66.3	5.7	136.0	12.7
	SD	0.0	0.0	1.5	0.0	7.1	0.6	8.9	0.6
6.4	1	12	3	31	3	60	5	116	13
	2	12	3	30	3	66	5	100	12
	3	12	3	31	3	60	6	119	13
	Mean	12.0	3.0	30.7	3.0	62.0	5.3	111.7	12.7
	SD	0.0	0.0	0.6	0.0	3.5	0.6	10.2	0.6
20	1	12	3	23	3	46	8	65	12
	2	12	3	27	3	46	7	77	10
	3	12	3	25	3	48	6	70	10
	Mean	12.0	3.0	25.0	3.0	46.7	7.0	70.7	10.7
	SD	0.0	0.0	2.0	0.0	1.2	1.0	6.0	1.2
64	1	12	3	23	9	28	10	33	11
	2	12	3	25	5	32	8	35	8
	3	12	3	23	8	32	7	32	10
	Mean	12.0	3.0	23.7	7.3	30.7	8.3	33.3	9.7
	SD	0.0	0.0	1.2	2.1	2.3	1.5	1.5	1.5
200	1	12	3	19	6	19	10	21	10
	2	12	3	19	8	20	11	20	12
	3	12	3	23	6	23	8	22	9
	Mean	12.0	3.0	20.3	6.7	20.7	9.7	21.0	10.3
	SD	0.0	0.0	2.3	1.2	2.1	1.5	1.0	1.5
640	1	12	3	14	6	14	8	14	9
	2	12	3	14	8	14	9	12	8
	3	12	3	17	9	15	11	16	12

Nominal concentration of formulation (µg/L)	Vessel no.	Frond number (#F) and colony number (#C) per test vessel							
		0 hr (day 0)		72 hr (day 3)		120 hr (day 5)		168 hr (day 7)	
		#F	#C	#F	#C	#F	#C	#F	#C
	Mean	12.0	3.0	15.0	7.7	14.3	9.3	14.0	9.7
	SD	0.0	0.0	1.7	1.5	0.6	1.5	2.0	2.1

Mean: arithmetic mean

SD: standard deviation

Table CP 10.2.1/04-3 Influence of the test item on *Lemna* growth: growth rates (r) and percentage inhibition of r

Nominal concentration of formulation (µg/L)	Growth rate r (1/day) and % inhibition of r					
	0-72 hr		0-120 hr		0-168 hr	
	r	%	r	%	r	%
Control	0.32	---	0.33	---	0.34	---
2.0	0.32	-1.6	0.34	-3.0	0.35	-1.2
6.4	0.31	1.7	0.33	1.0	0.32	7.1
20	0.24*	23.3	0.27*	18.1	0.25*	26.1
64	0.23*	28.9	0.19*	43.5	0.15*	57.4
200	0.17*	45.2	0.11*	67.4	0.08*	76.7
640	0.07*	77.1	0.04*	89.3	0.02*	93.9

% inhibition: increase in growth relative to that of control

*: mean value significantly lower than in control (according to a Dunnett-test, one-sided smaller, $\alpha = 0.05$)

Table CP 10.2.1/04-4 Influence of the test item on *Lemna* growth: areas under growth curves (AUC) and percentage inhibition of AUC

Nominal concentration of formulation (µg/L)	Areas under the growth curves (AUC) and % inhibition of AUC					
	0-72 hr		0-120 hr		0-168 hr	
	AUC	%	AUC	%	AUC	%
Control	696	---	2384	---	6504	---
2.0	708	-1.7	2484	-4.2	6764	-4.0
6.4	672	3.4	2320	2.7	5912	9.1
20	468*	32.8	1612*	32.4	3852*	40.8
64	420*	39.7	1148*	51.8	2108*	67.6
200	300*	56.9	708*	70.3	1132*	82.6
640	108*	84.5	236*	90.1	340*	94.8

% inhibition: increase in growth relative to that of control

*: mean value significantly lower than in control (according to a Dunnett-test, one-sided smaller, α = 0.05)

Table CP 10.2.1/04-5 Final biomass on the basis of dry weight (mg per test vessel) of *Lemna* plants after 7 days

Vessel no.	Nominal concentration of the formulation (µg/L)						
	Control	2.0	6.4	20	64	200	640
1	17.4	19.7	18.3	12.0	7.9	5.5	5.5
2	20.6	20.9	16.7	9.7	7.9	5.3	4.2
3	17.6	17.8	21.1	12.8	7.7	5.4	4.2
Mean	18.5	19.5	18.7	11.5*	7.8*	5.5*	4.6*
SD	1.8	1.6	2.2	1.6	0.1	0.1	0.8
% inhibition#	---	-5.3	-0.9	39.9	60.7	74.1	78.8

Mean: arithmetic mean

SD: standard deviation

*: mean value significantly lower than in control (according to a Dunnett-test, one-sided smaller, α = 0.05)

#: % inhibition based on the increase in biomass (mean final dry weight minus starting dry weight). Dry weight of three colonies with in total 12 fronds at test start: 0.09 mg

% inhibition: increase in dry weight relative to the control value

The EC_x concentrations determined in the study are presented below. The NOEC and LOEC for growth rate, areas under the growth curve and final biomass was determined to be 6.4 and 20 µg/L, respectively.

Table CP 10.2.1/04-6 Effect concentration values (day 0-7)

Parameter	7-day EC_{10} (µg/L) (95% confidence limits)	7-day EC_{50} (µg/L) (95% confidence limits)	7-day EC_{90} (µg/L) (95% confidence limits)
Growth rate	8.3 (5.4 – 12)	57 (45 – 73)	397 (278 – 629)
Area under growth curve	5.6 (2.3 – 10)	39 (26 – 61)	276 (156 – 673
Final biomass	6.3 (0.1 – 20)	67 (21 – 290)	>640 (n.d.)

n.d. could not be determined

III. Conclusion

In a 7-day growth inhibition study, *Lemna gibba* (duckweed) were exposed to JAU 6476 160 EC & KWG 4168 300 at nominal concentrations of 2.0, 6.4, 20, 64, 200 and 640 µg/L under semi-static conditions.

The 7-day NOEC was determined to be 6.4 µg/L. The 7-day E_rC_{50} value based on growth rate was determined to be 57 µg/L (equivalent to 17.0 µg spiroxamine/L and 9.18 µg prothioconazole/L, respectively). The EC_{50} based on area under growth curves (AUC) and final biomass were 39 and 67 µg/L, respectively.

Assessment and conclusion by applicant:

The study was conducted to the OECD 221 test guideline and the validity criteria according to the most recent 2006 version of the guideline are the same and have been met.

- Doubling time of frond number in the control to be less than 2.5 days (60 h), corresponding to an average specific growth rate of 0.275/d (actual: 2.0 days)

It is noted that the analytical results were variable, particularly at the lowest test concentration and in the aged test media. However, the stock solutions used to dose the test system gave good recoveries (101 – 114%) therefore it is considered that the test system was correctly dosed. The variable analytical results were attributed to difficulties with the analytical method at the lowest level. It is also noted that the lowest test level of 2.0 µg/L where the largest variation occurred is below the NOEC achieved in this test and therefore the results at this test level are not considered to affect the overall outcome of this study. The results have been expressed in terms of the nominal test concentrations which is considered to be appropriate for a formulation study with more than one active substance.

The study is considered to be acceptable.

The 7-day E_rC_{50} value based on growth rate was determined to be 57 µg/L (equivalent to 17.0 µg spiroxamine/L and 9.18 µg prothioconazole/L, respectively).

Data Point:	KCP 10.2.1/24
Report Author:	[redacted]
Report Year:	2020
Report Title:	Calculation of EC10, EC20 and EC50 values for Lemna gibba with prothioconazole 160 EC & spiroxamine 300 in a Lemna sp. growth inhibition test
Report No:	047836-EC037
Document No:	M-076044-01-1
Guideline(s) followed in study:	None
Deviations from current test guideline:	None
Previous evaluation:	No, not previously submitted -
GLP/Officially recognised testing facilities:	No, not conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The report M-077038-01-1 on the effects of exposure to Prothioconazole 160 EC & Spiroxamine 300 on the growth of *Lemna gibba* did not provide estimates of EC_{20} values. Therefore, these values as well as EC_{10} and EC_{50} values have been calculated in accordance with the Annex to Com. Reg. 283/2013.

The resulting EC_{10}, EC_{20} and EC_{50} values for yield (frond number) at 7 d were 3.93, 6.88 and 21.09 µg formulation/L, respectively. For growth rate (frond number) after 7 d, the EC_{10}, EC_{20} and EC_{50} values were 7.13, 14.31 and 54.21 µg formulation/L, respectively.

The resulting EC_{10}, EC_{20} and EC_{50} values for yield (dry weight) at 7 d were 3.76, 9.24 and 51.41 µg formulation/L, respectively. For growth rate (dry weight) after 7 d, the EC_{10}, EC_{20} and EC_{50} values were 9.99, 40.80 and 602.17 µg formulation/L, respectively.

I. Methods

The statistical evaluation was performed with statistical software ToxRatPro v3.3.0.

Effect concentrations with 10, 20 and 50% from the test item treatment when compared to the control were determined for yield and growth rate based on frond number and biomass (dry weight) after 7 days exposure. A Probit regression was performed, with confidence limits for the EC_x values estimated according to Fieller's theorem.

II. Results and Discussion

Yield (frond number) at 7 days

Regarding the calculation of EC_{10}, EC_{20} and EC_{50} values for yield (frond number) at 7 d, a statistically significant concentration/response was found (p(F) <0.001) for this parameter.

The resulting EC_{10}, EC_{20} and EC_{50} values and the respective confidence intervals are represented in the following table below.

Table CP 10.2.1/24-1 Results of the Probit analysis of yield (frond number) at 7 d: Selected effective concentrations (EC_x) of the test item and their 95%-confidence limits

Parameter	Yield [µg formulation/L]		
	EC_{10} (95 % confidence interval)	EC_{20} (95 % confidence interval)	EC_{50} (95 % confidence interval)
Effect on yield (frond number) at 7 d	3.93 (2.97 – 4.91)	6.88 (5.59 – 8.15)	20.09 (17.80 – 22.66)

The resulting EC_{10}, EC_{20} and EC_{50} values of 3.93 (95%CL: 2.97 – 4.91), 6.88 (95%CL: 5.59 – 8.15) and 20.09 (95%CL: 17.80 – 22.66) µg formulation/L, respectively, meet the goodness of fit criteria showing a significant concentration/response relationship, and therefore the estimated EC_x values are considered reliable.

Growth rate (frond number) at 7 days

Regarding the calculation of EC_{10}, EC_{20} and EC_{50} values for growth rate (frond number) at 7 d, a statistically significant concentration/response was found (p(F) <0.001) for this parameter.

The resulting EC_{10}, EC_{20} and EC_{50} values and the respective confidence intervals are represented in the following table below.

Table CP 10.2.1/24-2 Results of the Probit analysis of growth rate (frond number) at 7 d: Selected effective concentrations (EC_x) of the test item and their 95%-confidence limits

Parameter	Growth rate [µg formulation/L]		
	EC_{10} (95 % confidence interval)	EC_{20} (95 % confidence interval)	EC_{50} (95 % confidence interval)
Effect on growth rate (frond number) at 7 d	7.13 (5.49 – 8.87)	14.31 (11.78 – 16.89)	54.21 (48.32 – 60.81)

The resulting EC_{10}, EC_{20} and EC_{50} values of 7.13 (95%CL: 5.49 – 8.87), 14.31 (95%CL: 11.78 – 16.89), and 54.21 (95%CL: 48.32 – 60.81) µg formulation/L, respectively, meet the goodness of fit criteria showing a significant concentration/response relationship, and therefore the estimated EC_x values are considered reliable.

Yield (dry weight) at 7 days

Regarding the calculation of EC_{10}, EC_{20} and EC_{50} values for yield (dry weight) at 7 d, a statistically significant concentration/response was found (p(F) <0.001) for this parameter.

The resulting EC_{10}, EC_{20} and EC_{50} values and the respective confidence intervals are represented in the following table below.

Table CP 10.2.1/24-3 Results of the Probit analysis of yield (dry weight) at 7 d: Selected effective concentrations (EC_x) of the test item and their 95%-confidence limits

Parameter	Yield [µg formulation/L]		
	EC_{10} (95 % confidence interval)	EC_{20} (95 % confidence interval)	EC_{50} (95 % confidence interval)
Effect on yield (dry weight) at 7 d	3.76 (1.53 – 6.73)	9.24 (4.83 – 14.37)	51.41 (36.78 – 72.20)

The resulting EC_{10}, EC_{20} and EC_{50} values of 3.76 (95%CL: 1.53 – 6.73), 9.24 (95%CL: 4.83 – 14.37) and 51.41 (95%CL: 36.78 – 72.20) µg formulation/L, respectively, meet the goodness of fit criteria showing a significant concentration/response relationship, and therefore the estimated EC_x values are considered reliable.

Growth rate (dry weight) at 7 days

Regarding the calculation of EC_{10}, EC_{20} and EC_{50} values for growth rate (dry weight) at 7 d, a statistically significant concentration/response was found ($p(F) < 0.001$) for this parameter.

The resulting EC_{10}, EC_{20} and EC_{50} values and the respective confidence intervals are represented in the following table below.

Table CP 10.2.1/24-4 Results of the Probit analysis of growth rate (dry weight) at 7 d: Selected effective concentrations (EC_x) of the test item and their 95%-confidence limits

Parameter	Growth rate [µg formulation/L]		
	EC_{10} (95 % confidence interval)	EC_{20} (95 % confidence interval)	EC_{50} (95 % confidence interval)
Effect on growth rate (dry weight) at 7 d	9.99 (4.55 – 17.15)	40.80 (25.42 – 58.20)	602.17 (416.43 – 991.41)

The resulting EC_{10}, EC_{20} and EC_{50} values of 9.99 (95%CL: 4.55 – 17.15), 40.80 (95%CL: 25.42 – 58.20) and 602.17 (95%CL: 416.43 – 991.41) µg formulation/L, respectively, meet the goodness of fit criteria showing a significant concentration/response relationship, and therefore the estimated EC_x values are considered reliable.

III. Conclusion

The resulting EC_{10}, EC_{20} and EC_{50} values for yield (frond number) at 7 days were determined to be 3.93, 6.88 and 20.09 µg formulation/L.

The resulting EC_{10}, EC_{20} and EC_{50} values for growth rate (frond number) at 7 days were determined to be 7.13, 14.31 and 54.21 µg formulation/L.

The resulting EC_{10}, EC_{20} and EC_{50} values for yield (dry weight) at 7 days were determined to be 3.76, 9.24 and 51.41 µg formulation/L.

The resulting EC_{10}, EC_{20} and EC_{50} values for growth rate (dry weight) at 7 days were determined to be 9.99, 40.80 and 602.17 µg formulation/L.

Assessment and conclusion by applicant:

The statistical re-evaluation of the data was conducted in order to complete the data set for EC_x values for yield and growth rate.

The lowest E_rC_{50} determined was 54.2 µg/L which is based on frond number after 7-days. This value shall be taken as the critical endpoint determined from this algal study.

The values determined in the re-evaluation work are considered to be fully valid.

For procedural reasons studies listed in the Table CP 10.2.1-1 below are included in the current dossier as available data or information previously submitted but not necessarily evaluated. However, these reports have been fully superseded by newer studies. Consequently, no summaries of the reports have been included in the dossier.

Table CP 10.2.1-1: Studies previously submitted and not relied upon for the risk assessment

Data Point	Document No.	Date	Title
KCP 10.2.1/05	M-000348-01-1	2004	Toxicity of JAU 6476 technical to the blue-green alga Anabaena flos-aquae
KCP 10.2.1/06	M-000954-01-1	2004	Toxicity of JAU 6476 technical to the saltwater diatom Skeletonema costatum
KCP 10.2.1/07	M-001051-01-1	2004	JAU 6476-Desthio - Acute toxicity to crayfish (Procambarus clarkii) under static-renewal conditions
KCP 10.2.1/08	M-001064-01-1	2004	Toxicity of JAU 6476 technical to the freshwater diatom Navicula pelliculosa
KCP 10.2.1/09	M-083055-01-1	2002	Desthio JAU 6476: A 96-hour flow-through acute toxicity test with the saltwater mysid (Mysidopsis bahia)
KCP 10.2.1/10	M-083057-01-1	2002	JAU 6476: A 96 hour flow-through acute toxicity test with the saltwater mysid (Mysidopsis bahia)
KCP 10.2.1/11	M-104709-01-1	2003	Acute toxicity of JAU 6476-Desthio to the fathead minnow (Pimephales promelas) under static-renewal conditions
KCP 10.2.1/12	M-107321-01-1	2004	Acute toxicity of JAU 6476 technical to the Sheepshead minnow (Cyprinodon variegatus) under static-renewal conditions
KCP 10.2.1/13	M-266567-01-1	2006	Pseudokirchneriella subcapitata: growth inhibition test with prothioconazole-triazolylketone
KCP 10.2.1/14	M-266572-01-1	2006	Acute toxicity of JAU 6476-triazolylketone (tech.) to fish (Oncorhynchus mykiss) under static conditions
KCP 10.2.1/15	M-266597-01-1	2006	Acute toxicity of JAU 6476-triazolylketone (tech.) to the waterflea Daphnia magna in a static laboratory test system
KCP 10.2.1/16	M-000532-01-1	2004	Toxicity of JAU 6476 technical to duckweed (Lemna gibba G3) under static-renewal conditions
KCP 10.2.1/17	M-104599-01-1	2003	Toxicity of JAU 6476-Desthio to duckweed (Lemna gibba G3) under static-renewal conditions
KCP 10.2.1/18	M-291414-01-1	2007	Early life stage toxicity of prothioconazole technical to the rainbow trout (Oncorhynchus mykiss) under flow through conditions
KCP 10.2.1/19	M-279673-01-1	2006	Additional evaluation of the life-cycle toxicity test (report no. 200108) for prothioconazole-desthio (JAU 6476-desthio, M04) with the fathead minnow (Pimephales promelas) concerning the reproduction performance and the evaluation of pote
KCP 10.2.1/20	M-001562-01-1	2004	Desthio JAU 6476: A flow-through life-cycle toxicity test with the fathead minnow (Pimephales promelas)

KCP 10.2.1/21	M-104620-01-1	2003	Desthio JAU-6476: A flow-through life-cycle toxicity test with the saltwater mysid (Mysidopsis bahia)
KCP 10.2.1/22	M-266605-01-1	2006	Chironomus riparius 28-day chronic toxicity test with JAU 6476-S-methyl in a water-sediment system using spiked water

CP 10.2.2 Additional long-term and chronic toxicity studies on fish, aquatic invertebrates and sediment dwelling organisms

No chronic studies using Prothioconazole & Spiroxamine EC 460 are available. The formulation reflects the toxicity of the individual active substances therefore chronic studies using the formulation were not considered to be required.

CP 10.2.3 Further testing on aquatic organisms

No additional data using Prothioconazole + Spiroxamine EC 460 are available or considered to be necessary.

Although not conducted with Prothioconazole + Spiroxamine EC 460, an aquatic mesocosm study using Spiroxamine EC 500 (M-304557-01-1) is available and has been included in the risk assessment here because this study and the endpoint that it provides are considered to be integral to the risk assessment of spiroxamine. The study has been summarized below along with a summary of the re-analysis study which has also been conducted in order to assess the mesocosm data against current requirements.

Data Point:	KCP 10.2.3/01
Report Author:	[redacted]
Report Year:	2008
Report Title:	Biological effects and fate of Spiroxamine EC 500 in outdoor mesocosm ponds simulating actual exposure conditions in agricultural use
Report No:	EBKWX091
Document No:	M-304557-01-1
Guideline(s) followed in study:	OECD Guidance Document Simulated Freshwater Lentic Field Tests (Outdoor Microcosms and Mesocosms), April 2006 Guidance Document on Testing Procedures for Pesticides in Freshwater Microcosms (SETAC-Europe Workshop, Monks Wood, UK, July 1991) Community-Level Aquatic System Studies Interpretation Criteria (2002) (Proceeding from the CLASSIC Workshop)
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and accepted RAR (2010)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The aim of the study was to determine the ecological effects of a simulated contamination with Spiroxamine EC 500 on different trophic levels (phytoplankton, zooplankton, macroinvertebrates and periphyton) in outdoor mesocosms as an aquatic model ecosystem for lentic aquatic freshwater systems with different trophic levels. The fate of the compound in the individual compartments (water body, sediment and macrophytes) was monitored simultaneously.

Three applications of the test substance (with a 7-day interval) were made and the study ran for 14 weeks post-application.

Analysis of the test substance (Spiroxamine EC 500) in the water column 4 hours after each of the three fungicide applications indicated that all microcosms received the intended doses. On average 82.7% of the intended dose were measured by the accompanying chemical analysis.

Due to the fact that the variability in concentration levels probably is higher during the first hours post application due to incomplete mixing, and the concentration of higher treatment levels can be measured with a higher accuracy, it was decided to express the treatment-related responses observed in terms of the intended treatment levels (1.0, 2.1, 4.4, 9.3, 19.4 µg a.s./L).

At the end of the experiment (Day 84) approximately all measured concentrations were below the Limit of quantification (0.1 µg a.s./L).

The estimated DT_{50} of Spiroxamine in the water phase was determined as 3.8 days.

The DT_{50}-value for the whole system of 7.2 days (water + macrophytes + sediment) has to be considered with caution, as the fate of the test substance in the sediment showed a fluctuating pattern.

The overall NOEAEC for this mesocosm study was set at 9.3 µg/L. All effects observed up to the highest dose level of 19.4 µg a.s. /L showed a fast recovery. Taking the fact into consideration that the highest concentration was investigated in one replicate only, the overall NOEAEC for this mesocosm study on Spiroxamine was set at 9.3 µg a.s./L.

The taxa and species, on which the most pronounced effects were observed at this concentration are the Rotatoria. The most sensitive Rotatorian species were Asplancha, Polyarthra and Keratella quadrata. Similar sensitivities were observed for some species from several Phytoplankton families, for example the Cryptophyceae Chroomonas and Cryptomonas, the Diatomeae Achnantes and the Chlorophyceae Ankya judai and Characium, as well as the Chlorophyll a levels as a measure for the periphyton. The effects are statistically reflected in low indices for diversity, similarity and PRC community response.

Heterogeneous occurrence of filamentous algae was shown to be not compound dependent at concentrations up to 4.4 µg a.s./L by an additional laboratory study. Periphyton chlorophyll-a determinations revealed slight differences to the control at sampling days 28 to 42 in all dose levels. These differences to the control were not statistically significant at two consecutive measurement points. No indirect effects by potential adverse effects on periphyton were observed neither in zooplankton nor in phytoplankton.

Investigations by ASS and litter cages did not reveal any adverse effects for all dose levels within the entire test period.

No effects could be observed on the three macrophytes species, which were introduced into the ponds or occurred naturally.

First significant effects were detectable at 4.4 µg a.s./L. The study revealed a clear dose/response relationship. The highest observed effect classes belong to class 3B. No pronounced effects without recovery were observed within this study.

The overall NOEAEC for this mesocosm study (including the laboratory study with *filamentous algae*) was set at 9.3 µg/L.

I. Materials and Methods

Materials

Test Material	Spiroxamine EC 500
Lot/Batch #:	PF90087683
Purity:	49.8%, 501 g/L (content of a.s.)

Description:	Clear brown liquid
Stability of test compound:	Not reported
Reanalysis/Expiry date:	Not reported
Density:	1.006 g/mL
Treatments	
Test rates:	1.0, 2.1, 4.4, 9.3 and 19.4 µg a.s./L
Solvent/vehicle:	Water
Analysis of test concentrations:	Yes - days 0 7, 14, 18, 21, 28, 35, 42, 49, 56, 63, 70, 77 and 84
Test organisms	
Species:	Phytoplankton, zooplankton, macrozoobenthos and periphyton
Source:	Naturally occuring.
Acclimatisation period:	Mesocosms prepared 5 months prior to application
Test design	
Test vessel:	Cylindrical tanks made of black Polyethylene - each tank is 2.75 m in diameter and 1.55 m in depth, the surface is 5.94 m²
Test medium:	Tanks were filled with sediment to a level of about 14 cm and with water up to 1 m depth (The water was composed of 80 % local ground water and 20 % water from a nearby uncontaminated pond)
Replication:	3 replicates for the control, 2 replicates for the 1.0 – 9.3 µg a.s./L treatments and one replicate for the 19.4 µg a.s./L treatment.
Duration of test:	84 days
Environmental test conditions	
Temperature:	10.21 – 21.48 °C
Dissolved oxygen:	2.35 – 20.4 mg/L
pH:	Not reported
Photoperiod:	Natural. 0 – 9.70 hours sunshine per day

Study Design

The aim of the study was to determine the ecological effects of a simulated contamination with spiroxamine EC 500 on different trophic levels (phytoplankton, zooplankton, macroinvertebrates and periphyton) in outdoor mesocosms as an aquatic model ecosystem for lentic aquatic freshwater systems with different trophic levels. The fate of the compound in the individual compartments (water body, sediment and macrophytes) was monitored simultaneously.

The mesocosms used were twelve cylindrical tanks made of black Polyethylene (PE). They are installed next to the [redacted] Each tank is 2.75 m

in diameter and 1.55 m in depth, the surface is 5.94 m² (Figure 1 and Figure 2). When filled to the nominal operating depth of 1.0 m, each tank contains 5.94 m³ water. The respective water level is obtained from a gauge inside the tank. A plastic tray (depth: 0.2 m) is located on the bottom of each tank. The trays are filled with natural sediment up to a height of about 14 cm. The tanks are arranged with three basins each in four rows. All basins are connected with a separate 13th tank by pipes. During the months before the start of the study the water was pumped from the separate tank into the 12 study basins forwards and backwards, guaranteeing a homogenous mixing in the complete system in order to adjust the same chemical and biological conditions before study start. The ponds were disconnected from each other one week before the first application.

Twelve test tanks (6 m³ water, 1 m water depth) which were used in this study are especially designed systems which allow the establishment of almost identical conditions at the start of a study. The bottoms of the artificial tanks were covered with natural sediment (approximately 14 cm in height) five months prior to the study start. The water was composed of local ground water and water from a nearby uncontaminated pond which was inoculated several times with zooplankton from a natural pond nearby. Natural communities developed spontaneously from seeds and roots of aquatic plants as well as from air borne and naturally transferred stages of planktonic, benthic and filamentous algae organisms during the months before study start. Additionally one and two weeks respectively before application plants of three macrophyte species (Callitriche pallustris, Myriophyllum spicatum and Potamogeton crispus) were inserted into the ponds to initiate a heterogeneous habitat. In general, the artificial ponds are representative of a small stagnant water body.

The test substance was applied three times during the early growing season in May 2007 three times at an interval of 7 days onto the water surface of nine test ponds. The treatment levels were 1.0, 2.1, 4.4, 9.3 and 19.4 µg a.s./L (two replicates of 1.0 to 9.3 µg a.s./L, one replicate for 19.4 µg a.s./L). Three further tanks were used as untreated controls.

The mesocosms were investigated for a period of two weeks before and 14 weeks after treatment. Several times during the study period water, sediment and macrophyte samples were taken and analysed to investigate the concentration of the test substance in water and sediment. Further parameters evaluated were the taxonomic composition of phytoplankton, zooplankton and macroinvertebrates at different days before and after the applications.

All ponds had six predetermined sampling positions to cover the whole expanse. At these positions samples for biological samples and water were taken. The sediment samples were taken at different places which were chosen by chance each time. Separate equipment was used to sample controls, ponds with lower and ponds with higher concentrations of the test substance.

The water samples were taken with a commercial water-proof vacuum cleaner (Elektrostar, Starmix Zyklon HG 81). The suction tube (length: 120 cm, diameter 5 cm) was introduced vertically from the water surface down to about 15 cm above the sediment and withdrawn within a few seconds. During this time, approximately 12 L of water were filled into the sampling container.

Sediment samples were taken by means of a corer according to MILBRINK (1971) (sampled sediment surface: 19.6 cm², height about 10 cm). Previously it has been shown that chemicals primarily adsorb to the upper layer of the sediment, thus only the top about 2 cm of sediment were used for analysis. The sampled sedimentcolumn was filled into a glass-beaker having the dimension of the corer. The upper 2 cm of four separate sediment samples per pond were mixed.

During the study the abundance of macrophytes (% coverage) and filamentous algae was assessed visually. A qualitative statement on the development during the study is available in this report. At the end of the study all macrophytes and filamentous algae were harvested. The species were identified. For the determination of the biomass each species was dried in a dryer at 50°C.

250 mL of the mixed water sample was preserved with Lugol´s solution. For evaluation of these samples, a fixed volume of the thoroughly shaken phytoplankton sample were emptied into a sedimentation chamber (Utermöhlkammer) for phytoplankton identification in the laboratory and allowed to stand for

at least 12 hours. The identification and enumerating of the cells was made by means of a reversed microscope within five days after the filling. The number of enumerating fields (at least 5 fields) was chosen according to the actual concentration of algae. The number of individuals was calculated according to Utermöhl. Until identification and counting, the samples were stored at room temperature in the dark.

3.3 litre of water were sampled and merged from six sampling positions in each pond, resulting in 20 L water samples. The merged samples were filtered through a plankton sieve (mesh size: 56 µm) and preserved in a fixation solution (70% Ethanol containing 40 g Sucrose/L and 40 mL Glycerine/L). For species identification (see 4.1.2), the thoroughly shaken samples were filtered through a plankton sieve (mesh size: 30 to 50 µm). The sample bottles were rinsed again with fixation solution and emptied into the plankton sieve. The zooplankton was transferred into a petri-dish with enumeration lines, containing fixation solution. The zooplankton samples were evaluated by use of a reversed microscope, identifying and enumerating the individual organisms. To prevent evaporation the petri-dishes were covered. After determination, all samples were filled back into the bottles and stored at room temperature in the dark. If the density of organisms was too high, two methods were used to evaluate the sample. First, the individual sample was divided for enumerating by full evaluation of all sub-samples and summing up the results. Second, only a few sub samples were taken from a thoroughly homogenised sample and the enumerated results were projected for the total sample. Not all organisms were identified at the species level, only most abundant species and/or those which could be identified within a reasonable time frame.

Two artificial substrate samplers (ASS) per mesocosm were placed on the sediment surface. The ASS was pulled up at each sampling, placed in a bucket, and the animals were washed into the bucket with tap water. The water was passed through a 0.5 mm sieve, and the residue was fixed as described above for the zooplankton. The fixed samples were macroscopically examined (see 4.1.2) and enumerated under a binocular. Not all organisms were identified at the species level, only most abundant species and/or those which could be identified within a reasonable time frame.

Within this mesocosm study possible adverse effects on the detrivorous biocoenosis were investigated using litter cages. Small litter cages were filled with leaves of *Populus spec.* (about 5 g dry weight) and offered as habitat (cage size: diameter: 10 cm; high: 7 cm; mesh size 1 cm; stainless steel). The cages were exposed onto the sediment surface on small plates made of stainless steel mesh (diameter: 20 cm mesh size 0.02 cm). Three weeks before the first application eight cages were exposed in each pond, thus a natural detrivorous biocoenosis could develop onto the leaves until application. The dry weight of the Populus leaves was determined before exposure by weighing. Two and four weeks after the last application one cage per pond and six, eight and ten weeks after the last application two cages each respectively, were taken out of the ponds. The leaves were poured shortly with clean water, thereafter the water was passed through a 0.5 mm sieve, and the residue was fixed as described above for the zooplankton. The leaves were dried in a dryer at 50°C for at least 20 hours and the dry weight was determined.

One Emergence trap was fixed with lines at the center of each pond. The traps had a diameter of 56 cm (= 0.25 m²) In the traps, emerged organisms were fixed with 1,2-Ethandiol. The samples were preserved in fixation solution see above. As the ASS samples clearly show no effects on macroinvertebrates and as emerging organisms were not expected to be the most sensitive group, the samples of the emergence were not evaluated anymore.

The determination of chlorophyll-a was performed in accordance with NUSCH (DIN 3842 L 16 (DEV 1987)). Water samples were filtered through Whatman GF/C glass fibre filters (pore size 1 µm). The filters were folded, placed in aluminium foil and deep-frozen (-18°C) until extraction with ethanol. During the 2 hour extraction period the samples were agitated. The final extinction measurement was made in a 1-ray photometer (type MPM 1500, WTW) at a wavelength of 665 nm.

For the determination of the periphyton eight racks (made of stainless steel) per pond with ten glass slides each were placed in the water column (about 20 cm beneath the water surface. After a defined exposure time one slide per rack was taken out of the mesocosm and the periphyton on the glass slides

was wiped off with a glassfaser filter. The filters were folded, placed in aluminium foil and deep-frozen (- 18°C) until extraction with ethanol. During the 2 hour extraction period the samples were agitated. The final extinction measurement was made in a 1-ray photometer (type MPM 1500, WTW) at a wavelength of 665 nm.

The physico-chemical water parameters were measured several times during the study at an interval of about 7 days in mixed water samples.

For water analysis, 2 x 20 mL of a mixed water sample (see 4.3.4.2) was poured into a 50 mL amber glass bottle. On some occasions the water samples were obtained from three depths (ca. 10 – 30, 30 – 60 and 60 – 90 cm beneath water surface), to reveal the distribution of the test substance in the water column during the first 4days after application. For this purpose the water samples were obtained with a flask attached to a metal rod. The flask (1.0 L glass bottle) was moved around in the pond during filling to obtain water from different sites. Samples of the water in the control mesocosms were taken one hour before and 1 day after application to ensure that no cross contamination has entered the control ponds. Additionally to the water samples, the application suspensions were analysed on day 0, 7 and 14. For sediment analysis the upper 2 cm of four sediment samples (see 4.3.4.3) were mixed and about 320 g were taken for analysis.

As the heterogeneous occurrence of filamentous algae could not be sufficiently resolved within the mesocosm study, a separate laboratory study with focus on the filamentous algae only was performed in April 2008 using the same test regimes; three applications of 1.0, 2.1, 4.4, 9.3 and 19.4 µg a.s./L on Day 0, 7 and 14. The test was run for 21 days.

The biological data were analysed as follows: For each taxon (species up to phylum if appropriate; total counts per sample (e.g.: zooplankton, phytoplankton, sediment organisms) univariate statistics were used to test on differences between treatments and controls and to calculate a NOEC (No Observed Effect Concentration). At the community level, diversity and similarity indices as well as Principal Response Curves were used for analysis. The program Community Analysis V4.25 was used for all of the calculations, except for the Principal Response Curves. A former version of the CA program is described in (Hommen et al. 1992). The PRC analysis was performed with CANOCO 4.02 (DLO, Wageningen, NL), which represents the original program used in published papers describing the method.

Analytical method

Samples of water were analysed using the validated analytical method 00623, report reference M-031628-01-1 (**see Doc MCP Section 5**).

Samples of sediment were analysed using the validated analytical method 01088, report reference M-298750-01-1 (**see Doc MCP Section 5**).

Samples of macrophytes were analysed using the validated analytical method 00721, report reference M-304557-01-1 (**see Doc MCP Section 5**).

II. Results and Discussion

The analyzed concentrations of the application solutions gave an average of 92.1 % of the nominal concentrations for the three applications (minimum: 86 %, maximum: 97 %), thereby confirming nominal concentrations were achieved.

All analytical results correspond very well to each other (average over all concentrations at days 0/+4h, 7/+4h, 14/+4h: 82.7 %). The results demonstrate, that nominal concentrations had been initially applied at each of the three treatments. The concentration of the test substance in the pond water declined continuously. Four weeks after the last application the concentration at the two lowest treatment levels was below the limit of detection (= 0.102 µg a.s./L). In the highest treatment level the concentration in the water phase was below the limit of detection on day 70.

Stratified samples of three different water heights were taken to determine the a.s. distribution in the water column four hours, one and four days after each application. The results show that four hours after the second and third application the major part of the test substance was still in the upper water layer. 24 hours after the second and 4 days after the third application it was homogeneously distributed in the total water column. At the first application no stratification of the concentration of the test substance was found. Probably the rainfall after this application caused a faster distribution.

The measurement of centrifuged water samples did not reveal any significant difference to non-centrifuged water samples. Therefore it can be assumed, that the total amount of test substance was bio available.

The concentration of the test substance in the sediment increased until two weeks after the last application. Thereafter the concentrations decreased very slowly with a fluctuating pattern. The highest measured amount of the test substance in this matrix was 20.8 % of the nominal concentration on day 28 at the highest test concentration. At the end of the study the portion of the test substance in sediment was below 10 % of the initial applied amount for all treatments.

A very small portion of the applied substance was attributed to the macrophytes only (4 % as a maximum). Comparable to the water analysis the concentrations in the low dosages fall below the limit of quantification on day 28 and at the highest dosage on day 70.

The mean DT_{50}-value for dissipation of spiroxamine in the water is 3.77 days. The mean DT_{50}-value for the whole system (water plus macrophytes plus sediment) was 7.22 days. This latter value has to be considered with caution, as the fate of the test substance in the sediment was very heterogeneous.

Table CP 10.2.3/01-1 Summary of analysis of spray solutions used to dose the test system

Experimental day	Nominal concentration (mg a.s./L)	Mean measured concentration (mg a.s./L)	% nominal concentration
Day 0 [1 hour]	6.25	5.86	94
	13.13	12.07	92
	27.51	25.07	91
	58.15	52.06	90
	121.30	114.11	94
Day 7 [1 hour]	6.25	5.88	94
	13.13	12.42	95
	27.51	25.01	91
	58.15	49.95	86
	121.30	117.23	97
Day 14 [1 hour]	6.25	5.87	94
	13.13	12.50	95
	27.51	24.19	88
	58.15	50.95	88
	121.30	112.95	93
		Mean of %	92.1
		SD	1.9

Table CP 10.2.3/01-2 Mass balance of spiroxamine in the test system during the study – 1.0 µg a.s./L treatment

Experimental day	Measured concentration (as % of the total applied nominal amounts)			
	Water	Sediment	Macrophytes	Sum
0 [+ 4 hours]	72.5	-	-	72.5
1	53.7	-	-	53.7
4	24.9	-	-	24.9
7 [-1 hour]	15.5	12.0	0.003	27.5
7 [+ 4 hours]	94.5	-	-	94.5
8	62.2	-	-	62.2
11	35.7	-	-	35.7
14 [-1 hour]	28.6	12.4	0.404	41.2
14 [+ 4 hours]	76.5	-	-	76.5
15	60.5	-	-	60.5
18	21.9	-	-	21.9
21	12.3	6.33	0.000	19.6
28	6.51	13.4	0.000	20.9
42	3.74	12.2	0.000	16.3
56	3.82	5.85	0.000	9.71
70	3.84	12.1	0.000	16.0
84	3.78	6.61	0.000	10.5

Table CP 10.2.3/01-3 Mass balance of spiroxamine in the test system during the study – 2.1 µg a.s./L treatment

Experimental day	Measured concentration (as % of the total applied nominal amounts)			
	Water	Sediment	Macrophytes	Sum
0 [+ 4 hours]	67.2	-	-	67.2
1	53.4	-	-	53.4
4	25.3	-	-	25.3
7 [-1 hour]	16.0	3.24	0.420	19.7
7 [+ 4 hours]	83.7	-	-	83.7
8	63.4	-	-	63.4
11	33.4	-	-	33.4
14 [-1 hour]	21.2	12.2	1.18	34.5

Experimental day	Measured concentration (as % of the total applied nominal amounts)			
	Water	Sediment	Macrophytes	Sum
14 [+ 4 hours]	68.8	-	-	68.8
15	67.2	-	-	67.2
18	21.6	-	-	21.6
21	12.3	8.60	1.40	22.3
28	3.84	12.1	0.725	16.7
42	1.90	9.00	0.030	10.9
56	1.94	8.41	0.037	10.4
70	1.97	4.72	0.038	6.73
84	1.91	6.14	0.038	8.09

Table CP 10.2.3/01-4 Mass balance of spiroxamine in the test system during the study – 4.4 µg a.s./L treatment

Experimental day	Measured concentration (as % of the total applied nominal amounts)			
	Water	Sediment	Macrophytes	Sum
0 [+ 4 hours]	71.3	-	-	71.3
1	57.1	-	-	57.1
4	26.1	-	-	26.1
7 [-1 hour]	15.9	6.37	0.168	22.5
7 [+ 4 hours]	94.6	-	-	94.6
8	67.4	-	-	67.4
11	38.3	-	-	38.3
14 [-1 hour]	35.2	13.8	1.11	50.0
14 [+ 4 hours]	68.9	-	-	68.9
15	64.4	-	-	64.4
18	30.8	-	-	30.8
21	21.6	10.9	2.09	34.6
28	11.9	15.2	1.29	28.4
42	1.33	11.7	0.162	13.2
56	0.82	8.14	0.130	9.09
70	0.83	8.36	0.015	9.20
84	0.81	8.36	0.015	9.19

Table CP 10.2.3/01-5 Mass balance of spiroxamine in the test system during the study – 9.3 µg a.s./L treatment

Experimental day	Measured concentration (as % of the total applied nominal amounts)			
	Water	Sediment	Macrophytes	Sum
0 [+ 4 hours]	80.3	-	-	80.3
1	60.9	-	-	60.9
4	28.4	-	-	28.4
7 [-1 hour]	17.6	4.37	0.288	22.2
7 [+ 4 hours]	98.7	-	-	98.7
8	77.8	-	-	77.8
11	43.0	-	-	43.0
14 [-1 hour]	33.5	6.08	1.48	41.0
14 [+ 4 hours]	87.4	-	-	87.4
15	70.6	-	-	70.6
18	36.3	-	-	36.3
21	21.2	14.2	2.25	37.7
28	14.5	8.49	1.19	24.2
42	1.51	4.15	0.196	5.86
56	0.392	7.94	0.196	8.53
70	0.396	9.34	0.050	9.79
84	0.387	5.38	0.051	5.82

Table CP 10.2.3/01-6 Mass balance of spiroxamine in the test system during the study – 19.4 µg a.s./L treatment

Experimental day	Measured concentration (as % of the total applied nominal amounts)			
	Water	Sediment	Macrophytes	Sum
0 [+ 4 hours]	85.3	-	-	85.3
1	62.9	-	-	62.9
4	29.2	-	-	29.2
7 [-1 hour]	19.3	6.80	0.16	26.3
7 [+ 4 hours]	115.7	-	-	115.7
8	80.1	-	-	80.1
11	45.1	-	-	45.1
14 [-1 hour]	36.4	8.54	1.23	46.2

Experimental day	Measured concentration (as % of the total applied nominal amounts)			
	Water	Sediment	Macrophytes	Sum
14 [+ 4 hours]	86.0	-	-	86.0
15	87.9	-	-	87.9
18	53.8	-	-	53.8
21	46.3	5.85	3.68	55.9
28	12.1	20.8	1.92	34.9
42	1.83	13.9	0.15	15.9
56	0.377	13.0	0.089	13.4
70	0.185	12.4	0.029	12.6
84	0.180	8.30	0.030	8.51

Direct effects of the applications of Spiroxamine EC 500 to the overall community metabolism could not be observed. In the second half of the study the development of the filamentous algae caused, especially for one replicate of the control and one replicate of 1.0 µg/L, significant lower pH values and oxygen concentrations. The other treated ponds displayed slight higher values as compared to the control range during this period. All other investigated chemical physical parameters did not show any difference between treated ponds and the controls.

The macrophytes showed a strong growth in all ponds without any sign of a treatment related effect.

The measurements of the chlorophyll a content of the pelagial water revealed a short term effect for the three highest test concentrations between days 7 to 14. These findings are in agreement with the observed effects of the phytoplankton during this time period.

The periphyton was determined indirectly via chlorophyll a measurement. The total development was comparable for all ponds with a strong increase during the pre-treatment and a decrease after the application phase. A transient effect between days 28 and 42 cannot be excluded for the treated ponds, although one replicate of 4.4 and 9.3 µg/L each, was always in the range of the control. A statistical significant difference was calculated for day 42 only. A full recovery could be stated for all treatments on day 56 (= 6 weeks after last application). Periphyton consists of epiphytic algae, which to a lower amount can be detected in the pelagial water as well. The epiphytic algae, which were detected in the pelagial water in this study revealed a NOEC of 2.1 µg a.s./L for Achnantes spec., which was one of the dominant taxa in this study. For two other epiphytic taxa, which occurred in minor abundances only, a slight benefit was found at the two highest test concentrations.

The heterogeneous development of the filamentous algae in the mesocosm did not allow a reliable determination of this organism, as the values within the treated ponds were very inconsistent. Although the abundance of filamentous algae was lower in most of the treated ponds, a clear dose related effect could not be stated. Taking the heterogeneous growth of filamentous algae into consideration still a potential recovery for these algae up to the second highest concentration can be stated. A laboratory test performed to further investigate possible effects on the filamentous algae revealed a NOEC of 4.4 µg a.s./L. The results of this study are considered for the assessment of short term effects of the test substance to filamentous algae. Considering the results of the mesocosms and the additional laboratory study the NOEAEC was set to 9.3 µg/L.

The total results of the chlorophyll a, periphyton and filamentous algae result in a NOEAEC of 9.3 µg/L. The development of the periphyton and the filamentous algae obviously had no major influence on the dynamics of phyto- and zooplankton and the macroinvertebrates. The observed biological results for these groups are summarised in the following Table and in respective assessment for each treatment

level. Where statistically significant differences between treatments and controls were observed, and these were considered to be treatment-related and biologically significant, the responses were categorized in effect classes as mentioned in Working Document SANCO/3268/2001 rev.4(final), 2002, but following the adapted effect classes as described by Brock et al (2006) and De Jong et al. (2008). These effect classes are presented below.

Table CP 10.2.3/01-7 Effect classes used to evaluate the treatment-related responses of spiroxamine in the mesocosm study

Effects class	Definition of effects
1	Effect could not be demonstrated
2	Slight effect (minor in duration and magnitude)
3 A	Pronounced short-term effects and recovery within 8 weeks after the first application or total period of effects < 8 weeks followed by recovery
3 B	Pronounced short-term effects and recovery within 8 weeks after the last application followed by recovery
4	Pronounced effect but study measurement too short to demonstrate that treatment related effects last less than 8 weeks
5 A	Clear long-term effects lasting longer than 8 weeks but full recovery observed at end of experiment
5 B	Clear long-term effects lasting longer than 8 weeks but full recovery not observed at the end of the experiment

In the summary table (see below) trends of treatment-related effects are indicated by placing the effect class. A trend of an effect on a certain endpoint does not need to be statistically significant on consecutive sampling days or at the end of the experiment, but is considered relevant in connection with the overall effects observed.

Treatment level of 1.0 µg a.s./L: No consistent treatment-related effect could be observed for the population and community endpoints of the benthic macroinvertebrates sampled in the ASS and the phyto- and zooplankton studied.

Treatment level of 2.1 µg a.s./L: No consistent treatment-related effect could be observed for the population and community endpoints of the benthic macroinvertebrates sampled in the ASS and the phyto- and zooplankton studied.

Treatment-level of 4.4 µg a.s./L: The benthic macroinvertebrates were not affected by the treatments. For the zooplankton a slight adverse treatment-related effect became apparent for one taxonomic group the Rotatoria. Regarding the single species, only weak adverse effects which were statistically not significant were found for the dominant taxa *Polyarthra species* and *Asplanchna species*. But for the sum of Rotatoria a Class 2 effect resulted for a very short period after the third application (days 14 to 18). Another Rotatoria species (*Keratella quadrata*) showed higher densities as compared to the control after day 28. But it is questionable, if this finding is caused by lower competition of other zooplankter, as no biological significant adverse effect was found for the zooplankton anymore after day 21. Effects on the zooplankton community were not found. For the phytoplankton a Class 2 effect was found for one Diatomeae taxa (*Achnanthes species*) and a Class 3 effect for one Cryptophyceae taxa (*Cryptomonas species 20-30 µ*). These taxa mainly contributed to the total algae abundance, these effects were also reflected on the community level. However already 4 weeks after the last application a full recovery could be stated for all these endpoints.

Treatment-level of 9.3 µg a.s./L: Again no treatment related effect existed for the macroinvertebrates. The pronounced effects on the Rotatoria species *Asplanchna species* and *Polyarthra species* and thus on the sum of Rotatoria are regarded as Class 3 effect. But a recovery was obvious already 2 weeks after

the last application. The rotatoria dynamics caused a transient lower similarity of the zooplankton between the control and this dosage. A class 3 effect could also be derived from the PRC calculations. Nevertheless a recovery on the species level and the zooplankton community can be stated. The effects on single phytoplankton species and algae community, which were observed at 4.4 µg/L were also seen in this dosage although slightly more pronounced (Class 3 effect). The dynamics of the respective single taxa and the Community indices show a very high similarity between the control and this dosage from day 42 onwards. Regarding the results of the acroinvertebrates and the zoo- and phytoplankton this treatment can be considered as the overall NOEAEC$_{mesocosm}$.

Treatment-level of 19.4 µg a.s./L: The treatment-related responses observed in the microcosms treated with 19.4 µg a.s./L, and the species involved, were similar to the previous treatment but more pronounced. The strongest effects observed at this treatment level belong to class 3 thus this treatment could be considered as NOEAEC as well, but since only one replicate exist at this dose level, the concentration 9.3 µg a.s./l was chosen as the overall NOEAEC$_{mesocosm}$.

Table CP 10.2.3/01-8 Summary of treatment related effects observed in the mesocosm study in terms of effect classes

	Number of detected taxa *)	Test concentrations (µg a.s./L)				
		1.0	2.1	4.4	9.3	19.4
Zooplankton						
Cladocera	10 taxa	1	1	1	1	1
	Sum of Cladocera	1	1	1	1	2 ↑
Copepoda	2 taxa	1	1	1	1	1
	Naupli	1	1	1	1	2 ↓
Ostracada	1 taxa	1	1	1	1	1
Diptera	1 taxa	1	1	1	1	1
Rotatoria	14 taxa	1	1	1	1	1
	Asplanchna spec.	1	1	1	2 ↓	2 ↓
	Polyarthra spec.	1	1	1	3A ↓	3A ↓
	Keratella quadrata	1	1	3B ↑	3B ↑	3B ↑
	Sum of Rotatoria	1	1	2 ↓	3A ↓	3A ↓
Taxa richness		1	1	1	1	1
Diversity		1	1	1	1	1
Similarity		1	1	1	3B ↓	3B ↓
PRC community response		1	1	1	3B ↓	3B ↓
Macroinvertebrates						
(ASS)	46 taxa	1	1	1	1	1
Taxa richness		1	1	1	1	1
Diversity		1	1	1	1	2 ↑
Similarity		1	1	1	1	1
PRC community response		1	1	1	1	1

	Number of detected taxa *)	Test concentrations (µg a.s./L)				
		1.0	2.1	4.4	9.3	19.4
Littercages		1	1	1	1	1
Phytoplankton						
Cryptophyceae	1 taxa	1	1	1	1	1
	Chroomonas spec.	1	1	1	2 ↓	3A ↓
	Cryptomonas spec.	1	1	3A ↓	3A ↓	3A ↓
Diatomeae	8 taxa	1	1	1	1	1
	Achnantes spec.	1	1	2 ↓	3A ↓	3B ↓
Chlorophyceae	6 taxa	1	1	1	1	1
	Ankyra judai	1	1	1	2 ↑	2 ↑
	Characium spec.	1	1	1	2 ↑	2 ↑
Chrysophycea	2 taxa	1	1	1	1	1
Conjugatophyceae	3 taxa	1	1	1	1	1
Cyanobacteria	3 taxa	1	1	1	1	1
Euglenophyta	4 taxa	1	1	1	1	1
Taxa richness		1	1	1	1	2 ↓
Diversity		1	1	1	1	2 ↓
Similarity		1	1	1 ↓	3A ↓	3A ↓
PRC Community response		1	1	2 ↓	3A ↓	3A ↓
Chlorophyll a		1	1	2 ↓	2 ↓	2 ↓

*) only affected taxa are named (other taxa are summed up)

↓ = decrease

↑ = increase

III. Conclusion

Analysis of the test substance (Spiroxamine EC 500) in the water column 4 hours after each of the three fungicide applications indicated that all microcosms received the intended doses.

On average 82.7% of the intended dose were measured by the accompanying chemical analysis.

Due to the fact that the variability in concentration levels probably is higher during the first hours post application due to incomplete mixing, and the concentration of higher treatment levels can be measured with a higher accuracy, it was decided to express the treatment-related responses observed in terms of the intended treatment levels (1.0, 2.1, 4.4, 9.3, 19.4 µg a.s./L).

At the end of the experiment (Day 84) approximately all measured concentrations were below the Limit of quantification (0.1 µg a.s./L).

The estimated DT_{50} of Spiroxamine in the water phase was determined as 3.8 days.

The DT_{50}-value for the whole system of 7.2 days (water + macrophytes + sediment) has to be considered with caution, as the fate of the test substance in the sediment showed a fluctuating pattern.

The overall NOEAEC for this mesocosm study was set at 9.3 µg a.s./L. All effects observed up to the highest dose level of 19.4 µg a.s./L showed a fast recovery. Taking the fact into consideration that the highest concentration was investigated in one replicate only, the overall NOEAEC for this mesocosm study on spiroxamine was set at 9.3 µg a.s./L.

The taxa and species, on which the most pronounced effects were observed at this concentration are the Rotatoria. The most sensitive Rotatorian species were Asplancha, Polyarthra and Keratella quadrata.

Similar sensitivities were observed for some species from several Phytoplankton families, for example the Cryptophyceae Chroomonas and Cryptmonas, the Diatomeae Achnantes and the Chlorophyceae *Ankya judai* and Characium, as well as the Chlorophyll a levels as a measure for the periphyton.

The effects are statistically reflected in low indices for diversity, similarity and PRC community response.

Heterogeneous occurrence of filamentous algae was shown to be not compound dependent at concentrations up to 4.4 µg a.s./L by an additional laboratory study.

Periphyton chlorophyll-a determinations revealed slight differences to the control at sampling days 28 to 42 in all dose levels. These differences to the control were not statistically significant at two consecutive measurement points.

No indirect effects by potential adverse effects on periphyton were observed neither in zooplankton nor in phytoplankton.

Investigations by ASS and litter cages did not reveal any adverse effects for all dose levels within the entire test period.

No effects could be observed on the three macrophytes species, which were introduced into the ponds or occurred naturally.

First significant effects were detectable at 4.4 µg a.s./L. The study revealed a clear dose/response relationship. The highest observed effect classes belong to class 3B. No pronounced effects without recovery were observed within this study.

The overall NOEAEC for this mesocosm study (including the laboratory study with filamentous algae) was set at 9.3 µg a.s./L.

Assessment and conclusion by applicant:

The study was conducted to the guidance in place at the time of conduct.

Analytical measurements taken on the days of application demonstrate that the nominal test concentrations were achieved.

In accordance with the Aquatic Guidance Document, the study data has been re-evaluated to take account of MDD analysis as well as re-assessment of the effect classifications. The results of this re-assessment are presented in the subsequent summary of study (M-690576-01-1). Further commentary on the reliability and acceptability of these mesocosm data is also included at the end of the following study summary.

Data Point:	KCP 10.2.3/02
Report Author:	[redacted]
Report Year:	2019
Report Title:	Re-evaluation of a mesocosm study with spiroxamine
Report No:	E 413 3295-9
Document No:	M-690576-01-1
Guideline(s) followed in study:	None
Deviations from current test guideline:	None
Previous evaluation:	No, not previously submitted -
GLP/Officially recognised testing facilities:	not applicable
Acceptability/Reliability:	Yes

Executive Summary

The effects of the fungicide active substance spiroxamine on aquatic organisms of different trophic levels (phytoplankton, periphyton, zooplankton and macroinvertebrates), were investigated in an outdoor mesocosm study (Bayer CropScience AG Report ID EBKWX091) conducted in 2007, in accordance with the guidance available at that time. In the current 'Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters' EFSA (2013), it is suggested to report minimum detectable differences (MDD) in connection with the NOECs for taxa assessed in a micro- or mesocosm study. To derive a regulatory acceptable concentration (RAC), it is recommended that for at least eight populations of the sensitive taxonomic groups, the MDDs should be sufficiently low for an evaluation of direct effects. Therefore, the objective of this re-evaluation work was to calculate MDDs for the biological data sets, to determine for how many populations of sensitive groups a reliable evaluation of direct effects was possible, and to re-evaluate the effects according to the Aquatic Guidance Document (EFSA 2013).

Due to the fungicide mode of action of the test item, all taxonomic groups present in the mesocosms were considered for the re-evaluation. Therefore, the analysis was performed for the data sets of phytoplankton, periphyton, zooplankton and macroinvertebrates. NOECs and MDDs for the taxa considered for the evaluation were calculated using the one-sided Williams-test following the proposal outlined in Brock *et al.* (2015). In addition, the effects of the most relevant taxa were classified according to the current guidance and recommendations in order to allow an estimation of ETO- and ERO-RAC.

For phytoplankton, macroinvertebrates and zooplankton, 19 taxa plus pooled data on higher taxonomic levels fulfil the MDD criterion proposed by Brock *et al.* (2015). Furthermore, the chlorophyll a measurements of phytoplankton and periphyton as well as the macrophyte coverage could be evaluated. If a more strict criterion is applied, *e.g.* that the MDD should be at least once < 70% within the first four weeks after the first application, 13 taxa representing populations of macroinvertebrates, zooplankton and phytoplankton allow a statistical evaluation of direct effects: Tubificidae, Chironomini, *Chaobonus spec Simocephalus vetulus, Chydorus sphaericus, Eucercus lamellatus*, cyclopoid copepods (and nauplia larvae), *Polyarthra spec.*, *Chlamydomonas spec.*, coccoid Chlorophyceae, *Chroomonas spec.*, *Cryptomonas spec.* (20-30 µm), and Pennales (30-40 µm). Thus, the requirement of the Aquatic Guidance Document (EFSA PPR 2013) that the MDDs should be sufficiently low to allow the analysis of direct effect for at least 8 potential sensitive populations is met by the study.

The following effect classes were assigned to the different test concentrations:

- At the lowest test concentration of 1.0 µg/L, no treatment effects were found (class 1).
- At 2.1 µg/L, a slight direct effect on total phytoplankton abundance and a pronounced short-term promoting effect on the rotifer *Keratella quadrata* were detected (class 2 for the direct

effect on the phytoplankton and 3A if the potential temporary promotion of a rotifer species is considered an adverse effect). Other taxa showed no effects.

- At 4.4 µg/L, class 3A effects for total rotifers, total phytoplankton, chlorophyll a and *Cryptomonas spec*. were observed while some other taxa were slightly affected. Thus, the total effect class for 4.4 µg/L is considered 3A.
- At 9.3 µg/L, more taxa were affected, effects were more pronounced or prolonged and for two algae species, significantly higher abundances than in the controls were found at the end of the study. However, since in general both species were rare and no algae bloom was found at the end of the study, this was not considered to be ecologically relevant. Thus effect class 3A was chosen as the overall effect class for 9.3 µg/L.
- At 19.4 µg/L the effect classification was similar to the one for 9.3 µg/L. For leeches, higher abundances at the end of the study could not be excluded, which was considered as class 2/4A for the highest test concentration.

According to EFSA (2013) the ETO-RAC can be derived from the overall class 1 concentration of 1 µg/L (nominal for three applications) and would be 0.5 µg/L using the recommended assessment factor of 2 in EFSA (2013).

However, if the potential temporary promotion of the rotifer Keratella and the slight effect (class 2) on total phytoplankton is considered acceptable, the 2.1 µg/L concentration could be used to derive the ETO-RAC, considering that the observed effects here are of low ecological relevance.

Since rotifers and algae seem to be the most sensitive taxa while taxa with a lower recovery potential were found to be less sensitive (Cladocera, Copepoda, Chironomidae, Chaoboridae, Hirudinea, Oligochaeta) the study can also be used to derive an ERO-RAC. At 9.3 µg/L, no, slight or only effects with recovery within 8 weeks were found. Thus, the ERO-RAC can be derived using this concentration and would be 3.1 µg/L using an assessment factor of 3.

No clear long-term effects were found at the highest test concentration of 19.4 µg/L, only for a potential promotion of leeches the effect duration could not be assessed. Thus, the ERO-RAC would be 4.85 µg/L applying an assessment factor of 4 to consider the higher uncertainty due to data on leeches.

Table CP 10.2.3/02-1 Summary of the effect classification

Taxon or endpoint		1.0 µg/L	2.1 µg/L	4.4 µg/L	9.3 µg/L	19.4 µg/L
Zooplankton	Cladocera	1	1	2	2	2
	Daphnia Longispina	1	1	1	1	2+
	Simocephalus vetulus	1	1	1	1	2+
	Chydorus sphaericus	1	1	1	2+	2+
	Copepoda	1	1	1	1	2
	Cyclopoid Copepods	1	1	1	1	1
	Nauplii	1	1	1	1	3A
	Rotifera	1	1	3A	3A	3A
	Keratella quadrata	1	3A+	3A+	3A+	3A+
	Polyarthra spec.	1	1	1	3A+	3A+
	Asplanchna spec.	1	1	1	2	2
	Chaoborus spec. (larvae)	1	1	1	1	1
	Synchaeta spec.	1	1	2	2	2
Ma	Chironomidae	1	1	1	1	2

Taxon or endpoint		1.0 µg/L	2.1 µg/L	4.4 µg/L	9.3 µg/L	19.4 µg/L
	Chironominae	1	1	1	1	2
	Chironomini gen spec.	1	1	1	1	2
	Chaoborus crystallinus	1	1	1	1	1
	Hirundinea	1	1	1	1	2+/4A+
	Oligochaeta	1	1	1	1	1
Primary producers	Total phytoplankton	1	2	3A	3A	3A
	Cryptophyceae	1	1	2	3A	3A
	Chroomonas spec.	1	1	1	3A	3A
	Cryptomonas spec. 20-30 µm	1	1	3A	3A	3A
	Chlorophyceae	1	1	1	2	3A
	Chlamydomonas spec.	1	1	1	1	1
	coccoid Chlorophyceae	1	1	2	2	2
	Ankyra judayi	1	1	2A	3A+	3A+
	*Characium spec.**	1	1	1	2+/4A+	2+/4A+
	Closterium cf leibleinii*	1	1	1	3A+/4A+	3A+/4A+
	Diatoms (Bacillariophyceae)	1	1	1	1	2
	Achnanthes spec.	1	1	2	3A	3A
	Pennales 20-30 µm	1	1	1	1	1
	Cyanobacteria	1	1	1	1	1
	Pseudoanabaena spec.	1	1	1	1	1
	Phytoplankton chlorophyll a	1	1	3A	3A	3A
	Periphyton chlorophyll a	1	1	1	1	1
	Total macrophyte coverage	1	1	1	2	2
Proposed total effect class		**1**	**2/3A**	**3A**	**3A***	**3A/4A+**

Taxa set in bold indicate MDD category 1 taxa which are considered to present a potentially sensitive population.

A sign '+' indicates a promoting effect o

* Increased abundances at the end of the study on the two algae species were not considered an adverse effect, i.e. an algae bloom, since the species were not abundant.

I. Materials and Methods

In the outdoor mesocosm study, the test item (Spiroxamine EC 500) was applied three times (on day 0, 7 and 14 of the study). Effects were monitored for 84 days after the first application. The following biological examinations were performed during the study: the abundance of macrophytes (% coverage) and filamentous algae was assessed visually. Moreover, for the determination of the biomass, all macrophytes and filamentous algae were harvested at the end of the study. Determination of chlorophyll *a* (chl *a*) was performed for phytoplankton and periphyton. Phytoplankton was also sampled for identification and enumerating. The zooplankton was counted and identified at the species level, if possible. Emergence of insects was assessed by means of emergence traps. Macroinvertebrates were sampled by artificial substrates samplers (ASS). Additionally, macroinvertebrates were investigated using litter cages.

The test item was analysed in the water column and in the sediment during the study period. The limit of quantification (LOQ) of spiroxamine in the pond water was 0.102 µg/L. After each application (on day 0, 7 and 14), the test item dissipated continuously from the pond water. For the two lowest nominal test concentrations of 1.0 and 2.1 µg/L, the actual concentrations were below LOQ from day 42 onwards. At the higher nominal test concentrations of 4.4 and 9.3 µg/L, the measured concentration was below LOQ from day 56 onwards. After day 70, the actual concentration of the test item was below LOQ in all tanks (Figure 1).

For the re-evaluation analysis described in this report, the data sets used for the original statistical evaluation in the study results were provided by the sponsor as excel files. The input data sets are given in Annex 1. The following data sets were re-evaluated: zooplankton, macroinvertebrates in ASS, phytoplankton, phytoplankton and periphyton chlorophyll *a*, and macrophytes (% coverage). The development of filamentous algae was quantified using categories as described in the original study report. Since the Williams test used for calculation of NOEC and MDD assumes normal distributed data on an interval or ratio scale, these data were not re-evaluated. Note that for a better assessment of filamentous algae, a laboratory bioassay was performed and included in the original study report. In this laboratory study, short term effects occurred at 9.3 µg/L; thus the overall NOEC for filamentous algae in the laboratory study was stated to be 4.4 µg/L.

In this re-evaluation the same taxonomic groups were used, as in the original data sets following the recommendations of the guidance document (EFSA, 2013). Univariate statistics was performed on single species level (or the lowest taxonomic level identified) as well as on aggregated data like total abundances of organisms at a higher taxonomic level (*e.g.* family or order level) if these were provided.

The NOECs and related MDDs were calculated by means of the multiple t-tests of Williams (Williams, 1971, 1972). This test is similar to the well-known Dunnett-test (Dunnett 1955, 1964) but has slightly more power to detect differences to the controls (Jaki and Hothorn, 2013).

If the data did not show a monotonous dose-response relationship, the Williams´ test uses a moving average procedure before testing to achieve monotony. The assumption of a monotonous concentration-response can be made here because the focus is on direct effects on sensitive taxa.

The Williams´ tests were performed one-sided with $\alpha = 0.05$ (5% level of significance).

The abundance data of the organisms were log-transformed [y' = ln(a y+1)] before analysis, in order to approximate normality and homoscedasticity (homogeneity of variances) requirements (van den Brink *et al*. 2000). The factor 'a' was selected to achieve a logtransformed value close to 1 for the lowest non-zero value in the data set which results in log data sets scaled in a comparable way.

According to Brock *et al.* (2015), MDDs were calculated for the NOECs derived by the Williams´ test.

As abundance data were log-transformed for statistical testing, this MDD was also related to the transformed data, *i.e.* on a log-scale. Because % effects on a log-scale are difficult to interpret, the MDDs were transformed back to the abundance scale and these MDDs were used for evaluation (see Brock *et al*. 2015). Thus, for example, an MDD of 80 % means that the geometric mean abundance at the NOEC would have to be more than 80 % lower than the geometric mean of the controls to become statistically significant.

In the EFSA guidance document (EFSA 2013) no clear criteria are given for MDDs to be sufficiently low for a reliable analysis but classes for effect magnitudes have been proposed.

Table CP 10.2.3.02-2 Proposal on classes of MDDs due to treatment-related declines in abundance/biomass

Class	MDD	Comment
0	>100 %	No effect can be determined
I	90 – 100 %	Only large effects can be determined

Class	MDD	Comment
II	70 – 90 %	Large to medium effects can be determined
III	50 – 70 %	Medium effects can be determined
IV	<50 %	Small effects can be determined

Based on this MDD classification by EFSA, Brock *et al*. (2015) proposed a criterion for evaluating the MDDs per taxon. They suggest that for a reliable analysis of direct effects on a specific taxon, the following should be given:

- MDD < 100 % on at least 5 samplings or
- MDD < 90 % on at least 4 samplings or
- MDD < 70 % on at least 3 samplings or
- MDD < 50 % on at least 2 samplings after the application of the test item.

To apply this criterion, it was counted for each taxon how often (after the first application) the MDD fell in the MDD classes suggested by EFSA, 2013, *i.e.* how often the MDD was below 50 %, 70 %, 90 % and 100 %. Based on this count, it was decided if the MDD criterion proposed by Brock *et al*. (2015) was fulfilled.

Each taxon (or endpoint) was assigned to one of the three following categories, as suggested by Brock *et al*. (2015):

Category 1: The MDDs are sufficiently low for a reliable analysis according to the criterion proposed by Brock *et al*. 2015 (see above). These taxa are considered for the effect classification.

Category 2: The MDD criterion is not met, but on at least one sampling date (after application) a significant difference (negative or positive) to controls is found. These taxa are checked whether the statistical results indicate an effect of the treatment. If yes, the effects are classified

Category 3: The MDD criterion is not met and no significant deviation to control is found. These taxa are not further considered for evaluation

The biological effects were classified according to the recommendations of the EFSA aquatic guidance document (2013) and Brock *et al*. (2015) which are a modification of the scheme of De Jong *et al.* (2008) now considering also the MDD. In order to differentiate cases when recovery is clearly not shown from cases when recovery cannot be demonstrated because of *e.g.* the taxon is declining or absent in the controls during the recovery period, or the effect was found at the end of the study, or the MDD was too large to demonstrate recovery, such cases were also put into effect class 4 (originally used for cases when the study was too short to test recovery within 8 weeks). Therefore, class 4 was differentiated into 4A (study too short to analyse recovery) and 4B (recovery could not be assessed due to high MDD or decline of abundance also in the controls). If potential treatment effects were found at the end of the study, these were indicated as 2 - 4A or 3A - 4A because the duration of the effect could not be assessed.

Class 0 (treatment related effects cannot be statistically evaluated) does not fit well with the other effect classes because this is a property of the full data set for a taxon over all treatment levels including the controls while the other effect classes are related to the effect at the different treatment levels. Thus, if treatment-related effects on a taxon cannot be statistically evaluated, class 0 would apply for each treatment level. This is however covered already by the MDD categorisation: all taxa of MDD category 3 are the ones with effect class 0. Therefore, no effect classification was conducted for category 3 taxa.

With respect to the demonstration of 'full' or 'complete' recovery, EFSA (2013) states in footnote 33 (p. 121) that 'An endpoint is considered as recovered if the MDD allows statistical evaluation during the relevant recovery period (so excluding MDD class 0) and the conclusion of no statistically significant

effect between treated systems and controls is not caused by a decline of that endpoint in controls (*e.g.* at the end of the growing season). If these criteria are violated, a higher effect class has to be selected'.

This would mean that a difference to controls of *e.g.* 95 % can demonstrate full recovery as long as the MDD is < 100 % and the difference to control is not significant. This is in contradiction to the specific protection goals listed in the same document where only small effects over months, medium effects over weeks or large effects over days are considered acceptable for aquatic invertebrates (Table 14, p. 54 in EFSA 2013) while with respect to the MDD, small effects are defined as < 50 %, and large effects as 90 – 100 % (Table 31, p. 118 in EFSA (2013)).

Therefore, the more stringent recovery criterion of Brock *et al.* (2015) that 'recovery from treatment-related declines in abundance can only be considered if the MDDabu values during the relevant recovery period are < 70 % on at least one sampling, or < 90 % on at least two samplings, or if the % deviation from controls is less than 20 %' was used in this report.

The aim of this re-evaluation work was to provide data for deriving an ETO-RAC and an ERO-RAC according to the current EFSA aquatic guidance document (2013), *i.e.* to identify the treatment levels with effect classes up to 3A only based on the identification of the most sensitive taxa. Therefore, the focus of the effect evaluation was on the MDD category 1 taxa. Taxa of category 2 were only discussed if, based on the statistical finding, they were more sensitive than category 1 taxa. Category 3 taxa were not considered further because of high MDD values and missing statistical significance, and, in most cases, their low abundances.

Note that the MDD evaluation is related to direct effects, *i.e.* reduction of abundances. If a test item has an indirect effect shown as a treatment-related increase of abundance, the MDD classification is not applicable because the effects can be larger than 100 %. Thus, MDD category 2 taxa can be used for the assessment of indirect effect, even if MDDs are high. A promotion effect is indicated by a '+' sign added to the effect class, *e.g.* 3A+ indicates a pronounced but temporary promotion.

With hundreds of taxa and many sampling dates, a large number of statistical tests was conducted. Using an error level of 5 % means that a lot of positive findings were to be expected just by chance. In addition, by the default use of the Williams´ test as a most conservative multiple test, low NOECs can be obtained also in cases where there was no monotonous (or almost monotonous) concentration-response relation – just by the moving average procedure used by the Williams' test to achieve a monotonous concentration response before the testing. Therefore, the statistical findings were evaluated for their ecotoxicological relevance based on different criteria.

Table CP 10.2.3/02-3 Definition of effect classes based on EFSA (2013) and Brock *et al.* (2015)

Effect class	Description	Criteria
1	No treatment-related effects demonstrated	No (statistically and/or ecologically significant) effects observed as a result of the treatment Observed differences between treatment and controls show no clear causal relationship
2	Slight effects	Effects concern short-term and/or quantitatively restricted responses usually observed at individual samplings only.*
3A	Pronounced short-term effects (effect period < 8 weeks), followed by recovery	Clear response of sensitive endpoints, but full recovery within 8 weeks after the last application, or in the case of delayed responses and repeated applications, the duration of the effect period is less than 8 weeks and followed by full recovery.* Treatment-related effects demonstrated on consecutive samplings
3B	Pronounced effects longer than 8 weeks but recovery within 8 weeks after last application	Clear response of the endpoint in micro-/mesocosm experiment repeatedly treated with the test substance and that lasts longer than eight weeks (responses already start in treatment period), but full recovery of affected endpoint within eight weeks post last application.*
4A	Significant effects in short-term study	Clear effects (e.g. large reductions in densities of the population) observed, but the study is too short to demonstrate complete recovery within eight weeks after the (last) application. If delayed response is observed on the last sampling(s) only, this may be indicated as effect class 2-4A or 3A-4A.
4B	Significant short-term effects but MDD too high in recovery period	Significant short-term effects demonstrated but recovery cannot be properly evaluated due to high %MDD values in recovery period or the population in the controls is declining or even absent. If significant treatment-related response is demonstrated on one sampling but recovery cannot be interpreted due to high MDD this may be indicated as class 2-4B, in other case it can be 3A-4B.
5A	Pronounced long-term effect followed by recovery	Clear response of sensitive endpoint, effect period longer than 8 weeks and recovery did not yet occur within 8 weeks after the last application but full recovery is demonstrated to occur in the year of application.*
5B	Pronounced long-term effects without recovery	Clear response of sensitive endpoints (> 8 weeks post last application) and full recovery cannot be demonstrated before termination of the experiment or before the start of the winter period.

*Note that following Brock *et al.* (2015) recovery can only be considered if the MDDs during the recovery period are <70 % on at least one sampling or < 90 % on at least two samplings or if the deviation to controls is less than 20 %. If this is not the case, an appropriate higher class has to be selected.

The program Community Analysis (CA) V4 was used for NOEC, MDD and diversity calculations. A former version of the CA program is described in Hommen *et al.* (1994). Calculations of the CA program have been validated by means of example data and of calculations using MS-Excel™ (Microsoft® Corp.) and ToxRat® (Vers. 2.09).

II. Results and Discussion

Zooplankton

Total zooplankton, the sums for cladocerans, daphnids, copepods and rotifers but also several lower taxa fulfil the MDD criteria proposed by Brock *et al*. (2015). In total eight taxa representing potentially sensitive populations (*Daphnia longispina, Simocephalus vetulus, Chydorus sphaericus, Cyclopoida* (adults and copepods as well as nauplia larvae which could not be further determined), *Keratella quadrata, Polyarthra spec.*, *Asplanchna spec.* and *Chaoborus spec.*) could be evaluated for direct effects according to the MDD criterion proposed by Brock *et al.* (2015). Ten other taxa belonged to MDD category 2, *i.e.* the MDDs did not meet the criterion but significant deviations to controls were found at least once after the first application.

Table CP 10.2.3/02-4 % MDDs for the taxa in the zooplankton data set which met the criterion proposed by Brock *et al.* (2015). MDD category 1

Zooplankton	Summary			
	Min	Max	Mean	MDD Cat
Sum of Cladocerans	43	90	76	1
Sum of Daphnids	78	167	105	1
Sum of Copepods	47	88	73	1
Sum of rotifers	30	96	69	1
Daphnia longispina	84	167	106	1
Simocephalus vetulus	63	104	85	1
Chydorus sphaericus	56	114	94	1
Cyclopoid Copepods	58	91	80	1
Copepod Nauplii	50	88	72	1
Keratella quadrata	79	102	89	1
Polyarthra spec.	45	104	88	1
Asplanchna spec.	73	107	86	1
Chaoborus spec. larvae	62	213	103	1

MDD cat = category based on MDD evaluation according to Brock et al. (2015)

Table CP 10.2.3/02-5 % MDDs for the taxa in the zooplankton data set which met the criterion proposed by Brock *et al.* (2015). MDD category 2

Zooplankton	Summary			
	Min	Max	Mean	MDD Cat
Graptoleberis testudinaria	71	165	123	2
Acroperus harpae	105	166	122	2
Eucercus lamellatus	34	157	101	2
Ceriodaphnia reticulate	n.c.	n.c.	n.c.	2
Ostracodes	77	145	107	2
Testudinella patina	87	158	127	2

Zooplankton	Summary			
	Min	Max	Mean	MDD Cat
Lepadella patella	84	166	112	2
Synchaeta spec.	85	105	98	2
Hexarthra spec.	105	171	126	2
Chaoborus spec. pupae	225	246	232	2

MDD cat = category based on MDD evaluation according to Brock et al. (2015)

Significantly lower abundances than in the controls on at least two consecutive sampling dates were found for two rotifer species and also for the sum of rotifers. The most sensitive taxa according to the statistical analysis were *Asplanchna spec.* and *Polyarthra spec.* For *Asplanchna spec.*, NOECs of 4.4 and 2.1 µg/L were found on day 11 and 14 indicating potential short-term effects. However, abundance of *Asplanchna* collapsed in all mesocosms, including the controls during the first three weeks after the day of the first application. After the first and the third application, the decline in 19.4 µg/L was not stronger than in the controls and on day 14 there was no clear concentration response relation. Thus, effects of 9.3 and 19.4 µg/L were considered slight (conservatively, class 2 effects are proposed). The findings of *Asplanchna* later in the mesocosm treated with 19.4 µg/L supports that the interpretation of no pronounced effects on this species up to the highest test concentration.

Table CP 10.2.3/02-6 NOECs [µg/L] and related % MDDs (in brackets) for the taxa in the zooplankton data set

Macrozoobenthos		Days after application												
MDD cat	Taxa / day	-14	-7	0	4	7	11	14	18	21	28	42	56	70	84
1	Sum of Cladocerans	≥19.4 (84)	≥19.4 (91)	≥19.4 (89)	≥19.4 (73)	≥19.4 (85)	2.1-(47)	≥19.4 (83)	≥19.4 (85)	≥19.4 (73)	1+ (53)	9.3+ (78)	≥19.4 (90)	≥19.4 (85)	≥19.4 (89)
1	Sum of Daphnids	≥19.4 (122)	≥19.4 (103)	≥19.4 (100)	≥19.4 (100)	≥19.4 (100)	≥19.4 (101)	9.3+ (98)	≥19.4 (99)	≥19.4 (93)	≥19.4 (84)	2.1+ (78)	9.3-(84)	≥19.4 (85)	≥19.4 (85)
1	Sum of Copepods	≥19.4 (63)	≥19.4 (65)	≥19.4 (66)	≥19.4 (82)	≥19.4(81)	≥19.4(88)	≥19.4(72)	≥19.4 (78)	≥19.4(75)	≥19.4(76)	≥19.4(69)	9.3-(47)	≥19.4(73)	≥19.4(64)
1	Sum of Rotifers	≥19.4 (27)	≥19.4 (74)	1-(48)	≥19.4 (77)	4.4-(46)	4.4-(55)	<1-(30)	2.1-(66)	≥19.4 (71)	≥19.4 (96)	<1+ (76)	≥19.4 (84)	≥19.4 (84)	≥19.4 (79)
1	Daphnia longispina	≥19.4 (121)	≥19.4 (103)	≥19.4 (100)	≥19.4 (101)	≥19.4 (100)	≥19.4 (101)	≥19.4 (98)	≥19.4 (99)	≥19.4 (93)	≥19.4 (84)	2.1+ (84)	9.3-(84)	≥19.4 (155)	≥19.4 (167)
1	Simocephalus vetulus	≥19.4 (95)	≥19.4 (99)	≥19.4 (97)	9.3+ (69)	≥19.4 (81)	2.1-(63)	≥19.4 (88)	≥19.4 (92)	≥19.4 (97)	≥19.4 (80)	≥19.4 (97)	≥19.4 (89)	≥19.4 (104)	≥19.4 (74)
1	Chydorus sphaericus	≥19.4 (85)	≥19.4 (90)	≥19.4 (95)	≥19.4 (93)	≥19.4 (85)	≥19.4 (56)	4.4+ (80)	≥19.4 (98)	≥19.4 (98)	≥19.4 (101)	≥19.4 (107)	≥19.4 (114)	≥19.4 (103)	≥19.4 (104)
1	Cyclopoid Copepods	≥19.4 (78)	≥19.4 (55)	≥19.4 (63)	≥19.4 (86)	≥19.4 (88)	≥19.4 (85)	≥19.4 (91)	≥19.4 (89)	≥19.4 (74)	≥19.4 (77)	≥19.4 (87)	≥19.4 (61)	≥19.4 (83)	≥19.4 (58)
1	Copepod Nauplii	≥19.4 (63)	≥19.4 (74)	≥19.4 (75)	≥19.4 (82)	≥19.4 (72)	≥19.4 (88)	4.4+ (58)	≥19.4 (80)	≥19.4 (81)	≥19.4 (79)	9.3-(52)	9.3-(50)	≥19.4 (71)	≥19.4 (77)

Macrozoobenthos		Days after application													
MDD cat	Taxa / day	-14	-7	0	4	7	11	14	18	21	28	42	56	70	84
1	Keratella quadrata	≥19.4 (29)	≥19.4 (70)	≥19.4 (44)	≥19.4 (82)	≥19.4 (90)	≥19.4 (99)	≥19.4 (79)	≥19.4 (87)	≥19.4 (87)	≥19.4 (102)	1+ (92)	1+ (86)	1+ (90)	≥19.4 (85)
1	Polyarthra spec.	≥19.4 (95)	≥19.4 (86)	≥19.4 (72)	≥19.4 (72)	4.4+ (45)	4.4+ (67)	4.4+ (93)	9.3- (98)	4.4+ (95)	≥19.4 (101)	≥19.4 (104)	≥19.4 (97)	4.4+ (98)	≥19.4 (99)
1	Asplanchna spec.	≥19.4 (99)	≥19.4 (82)	<1- (50)	≥19.4 (85)	≥19.4 (80)	4.4+ (73)	2.1- (85)	≥19.4 (107)		9.3+ (n.c.)	9.3+ (n.c.)			
1	Chaoborus spec. larvae	9.3+ (87)	≥19.4 (169)	≥19.4 (145)	≥19.4 (62)	≥19.4 (114)	≥19.4 (80)	≥19.4 (111)	≥19.4 (92)	≥19.4 (89)	≥19.4 (106)	≥19.4 (95)	≥19.4 (89)	≥19.4 (213)	≥19.4 (79)
2	Graptoleberis testudinaria	≥19.4 (274)	≥19.4 (n.c.)	≥19.4 (159)	≥19.4 (157)	≥19.4 (n.c.)	≥19.4 (n.c.)	≥19.4 (165)	≥19.4 (146)	≥19.4 (160)	≥19.4 (113)	1+ (71)	≥19.4 (97)	≥19.4 (98)	≥19.4 (100)
2	Acroperus harpae		≥19.4 (142)	≥19.4 (161)		9.3+ (n.c.)	≥19.4 (n.c.)				≥19.4 (166)		≥19.4 (110)	≥19.4 (105)	≥19.4 (106)
2	Eucercus lamellatus		9.3+ (n.c.)	≥19.4 (113)	≥19.4 (119)	≥19.4 (127)	1- (75)	≥19.4 (101)	≥19.4 (103)	9.3+ (34)	≥19.4 (114)	≥19.4 (103)	<1- (80)	≥19.4 (100)	≥19.4 (157)
2	Ceriodaphnia reticulata				≥19.4 (n.c.)				9.3+ (n.c.)		≥19.4 (n.c.)	9.3+ (n.c.)			
2	Ostracodes	≥19.4 (198)	≥19.4 (n.c.)	≥19.4 (n.c.)		≥19.4 (n.c.)	≥19.4 (n.c.)	9.3+ (n.c.)	≥19.4 (145)	≥19.4 (167)	≥19.4 (112)	≥19.4 (77)	≥19.4 (94)	≥19.4 (94)	≥19.4 (93)
2	Testudinella patina						≥19.4 (n.c.)	≥19.4 (158)	≥19.4 (n.c.)	2.1+ (n.c.)	≥19.4 (137)	<1+ (148)	<1+ (125)	≥19.4 (105)	≥19.4 (87)
2	Lepadella patella				≥19.4 (n.c.)				≥19.4 (n.c.)	≥19.4 (166)	≥19.4 (137)	1+ (91)	≥19.4 (84)	≥19.4 (98)	≥19.4 (94)
2	Synchaeta spec.			9.3- (98)	≥19.4 (105)	≥19.4 (101)	≥19.4 (100)		4.4- (85)	≥19.4 (99)	≥19.4 (99)	≥19.4 (n.c.)	≥19.4 (n.c.)		≥19.4 (n.c.)
2	Hexarthra spec.										9.3- (n.c.)	≥19.4 (171)	≥19.4 (108)	≥19.4 (121)	≥19.4 (105)
2	Chaoborus spec. pupae		≥19.4 (n.c.)		≥19.4 (n.c.)	≥19.4 (n.c.)	≥19.4 (246)		≥19.4 (n.c.)	≥19.4 (n.c.)		≥19.4 (n.c.)	≥19.4 (225)	≥19.4 (225)	9.3+ (n.c.)

Signs indicate the direction of a significant effect and colours indicate the different NOECs. Blank fields: taxon not present. n.c.: MDD could not be calculated because of absence in the controls. Empty cells indicate absence in all samples of that day. Cat = MDD category according Brock et al. (2015).

Figure CP 10.2.3/02-1 *Asplanchna* spec.: Geometric means per treatment level and range of controls

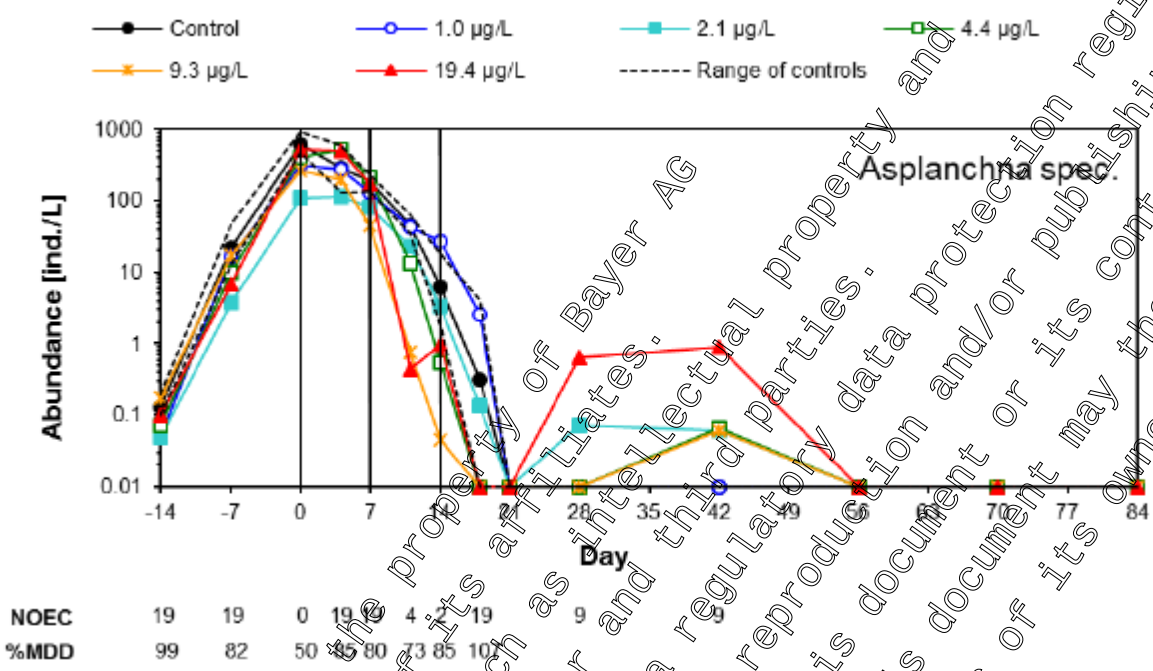


Figure CP 10.2.3/02-2 *Polyarthra* spec.: Geometric means per treatment level and range of controls

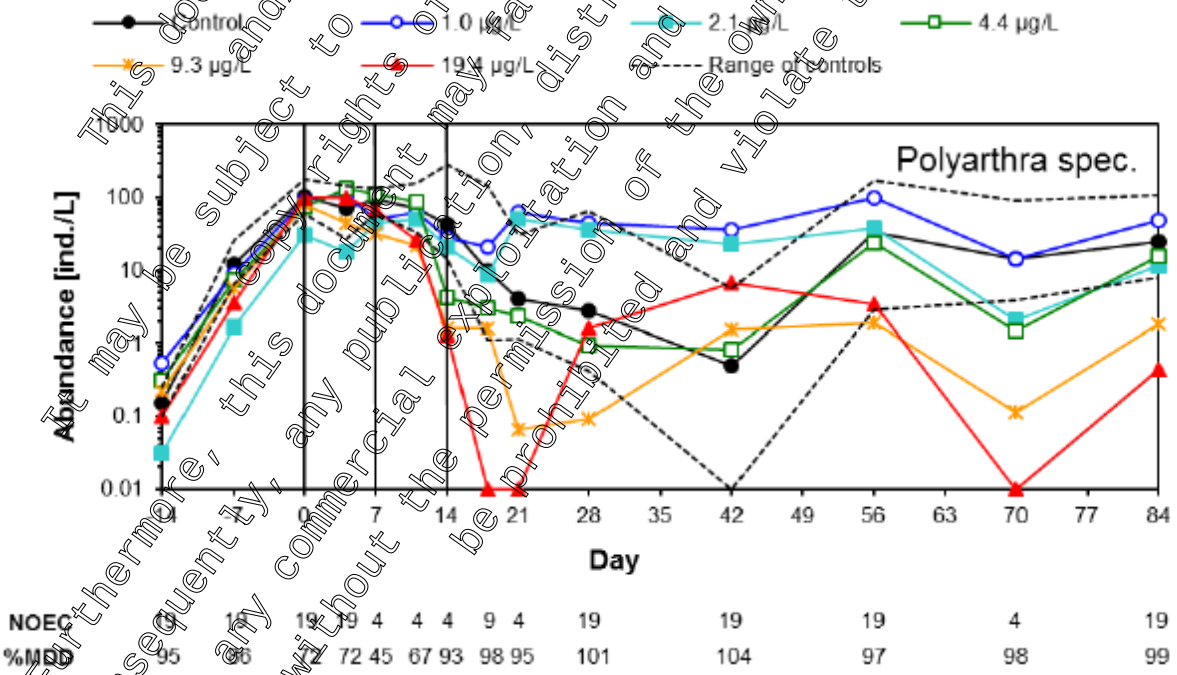


Figure CP 10.2.3/02-3 *Keratella quadrata*: Geometric means per treatment level and range of controls

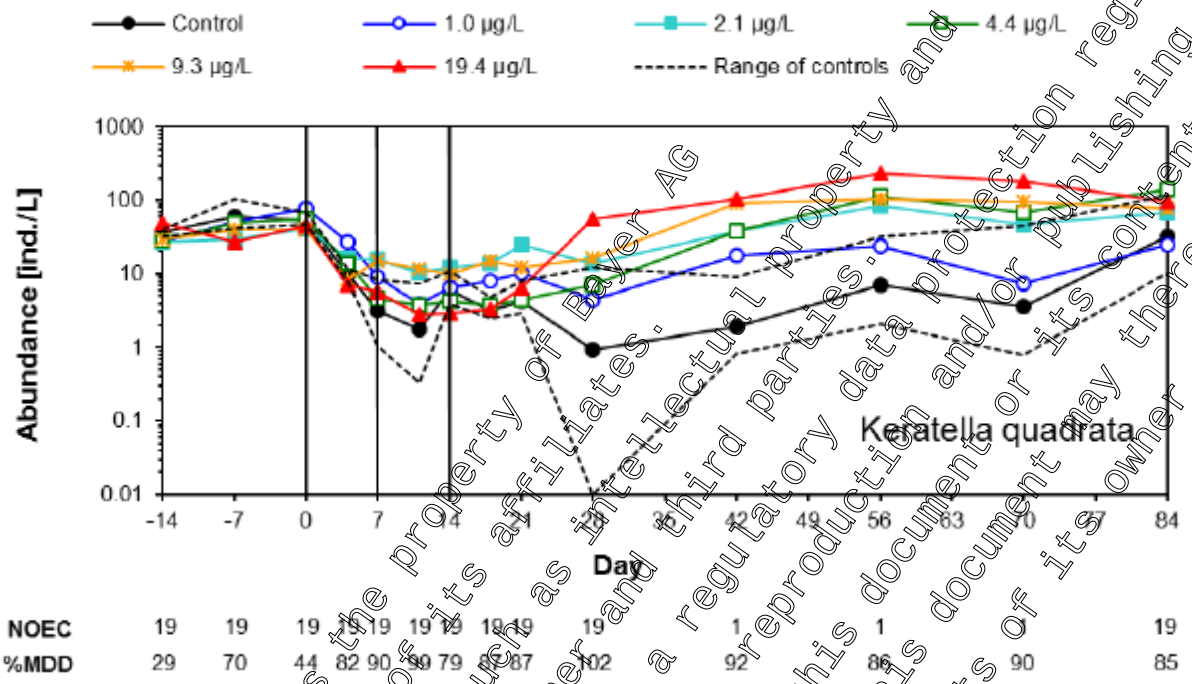


Figure CP 10.2.3/02-4 Sum of rotifers: Geometric means per treatment level and range of controls

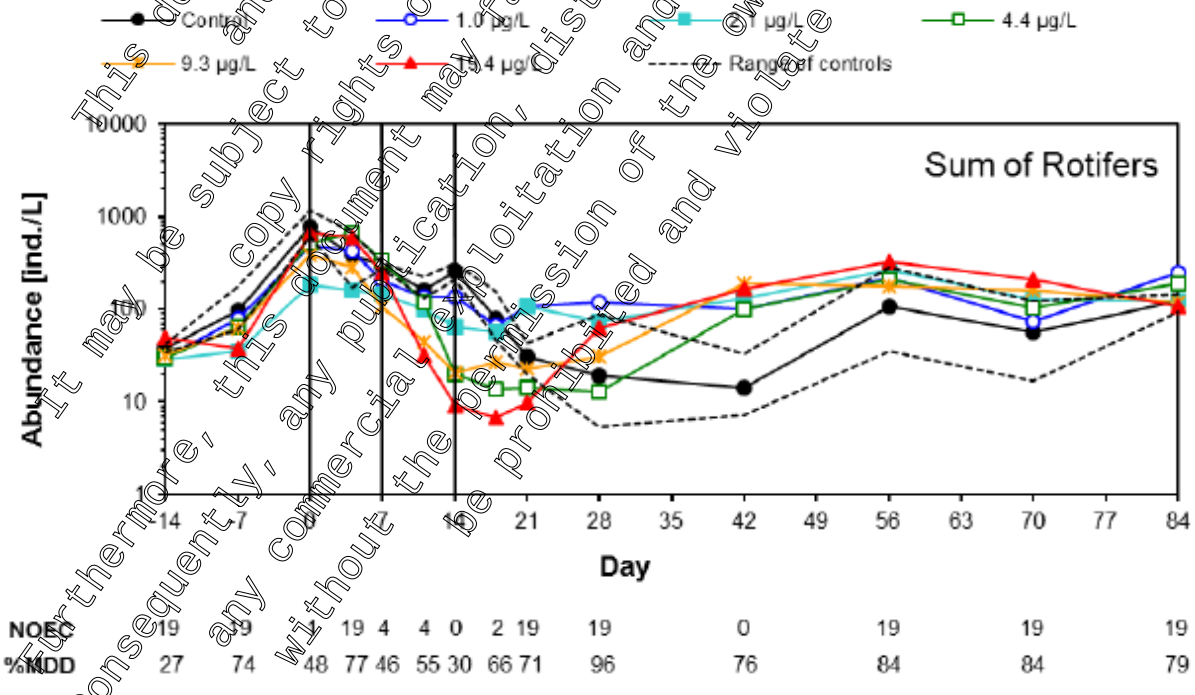
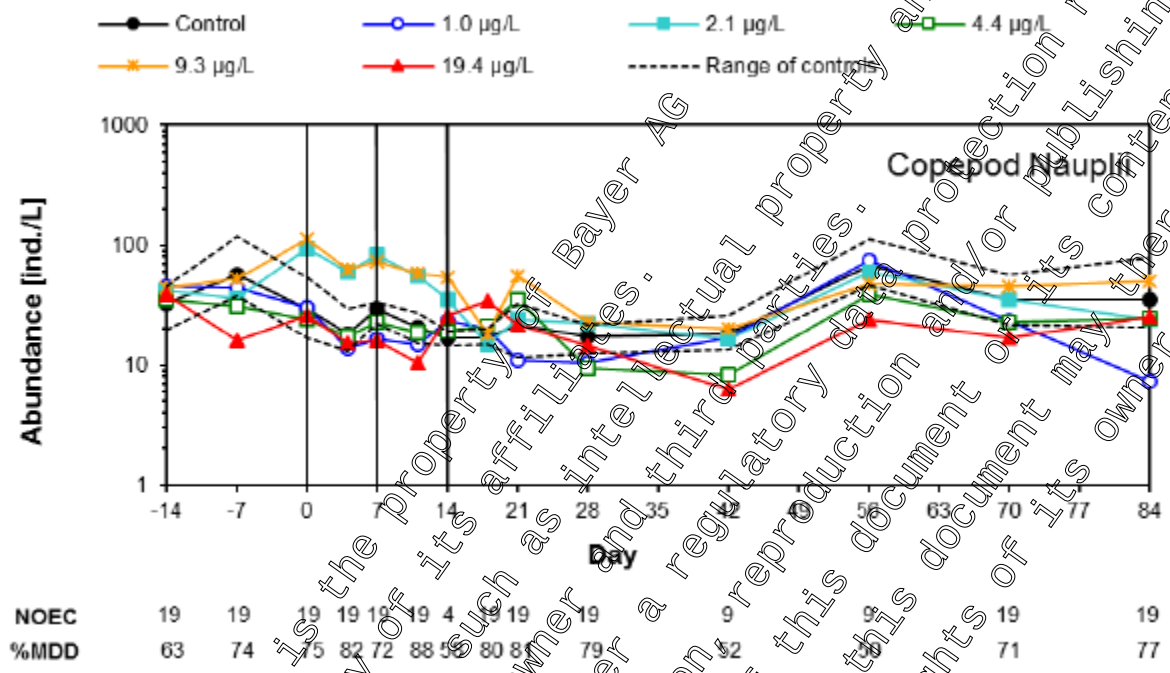
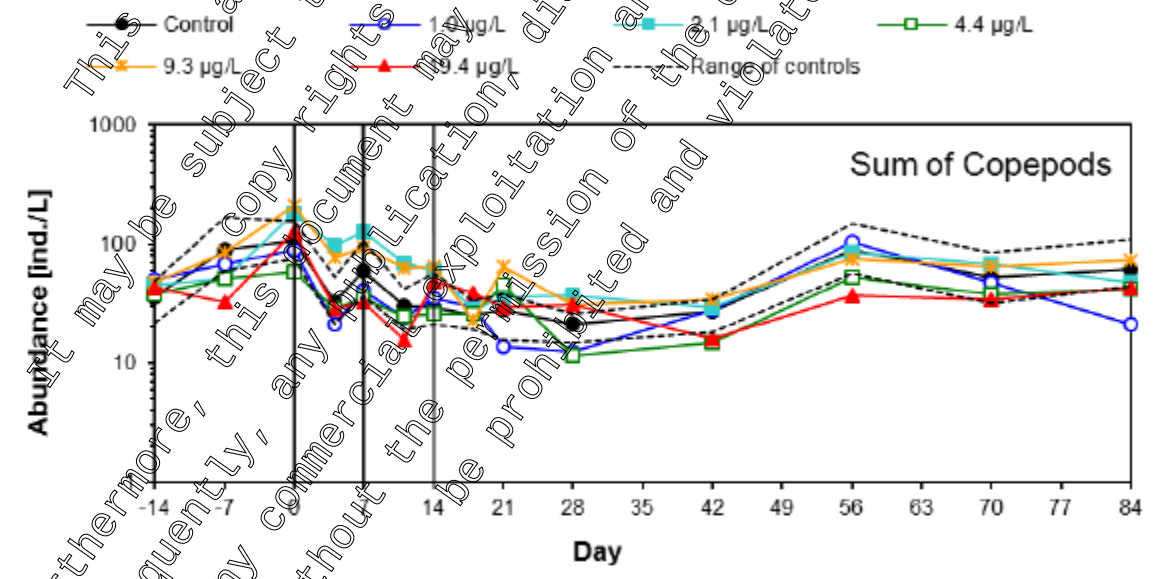


Figure CP 10.2.3/02-5 Copepod nauplii: Geometric means per treatment level and range of controls



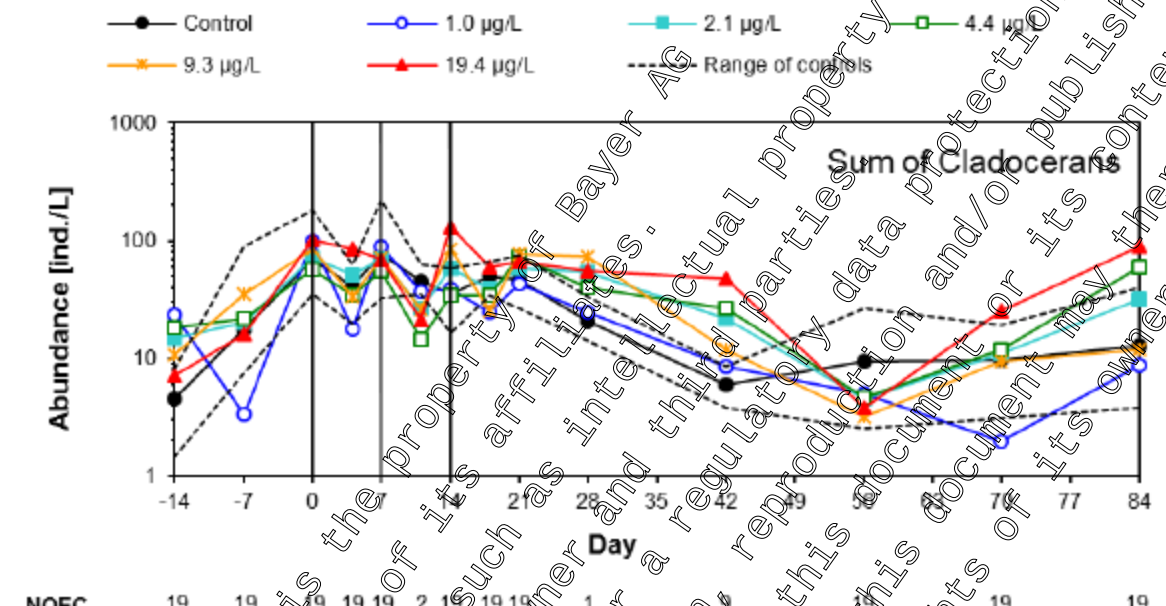
NOEC	19	19	19	19	19	19	4	19	19	19	9	9	19	19
%MDD	63	74	75	82	72	88	55	80	81	79	52	59	71	77

Figure CP 10.2.3/02-6 Sum of Copepods: Geometric means per treatment level and range of controls



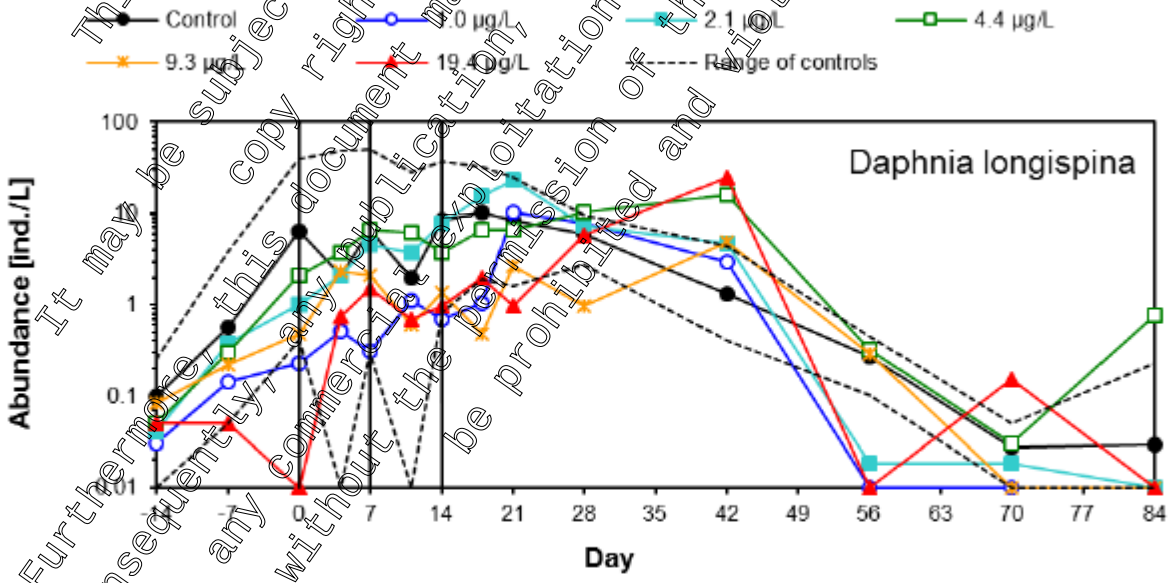
NOEC	19	19	19	19	19	19	19	19	19	19	19	9	19	19
%MDD	63	65	66	82	81	88	72	78	75	76	69	47	73	64

Figure CP 10.2.3/02-7 Sum of Cladocerans: Geometric means per treatment level and range of controls



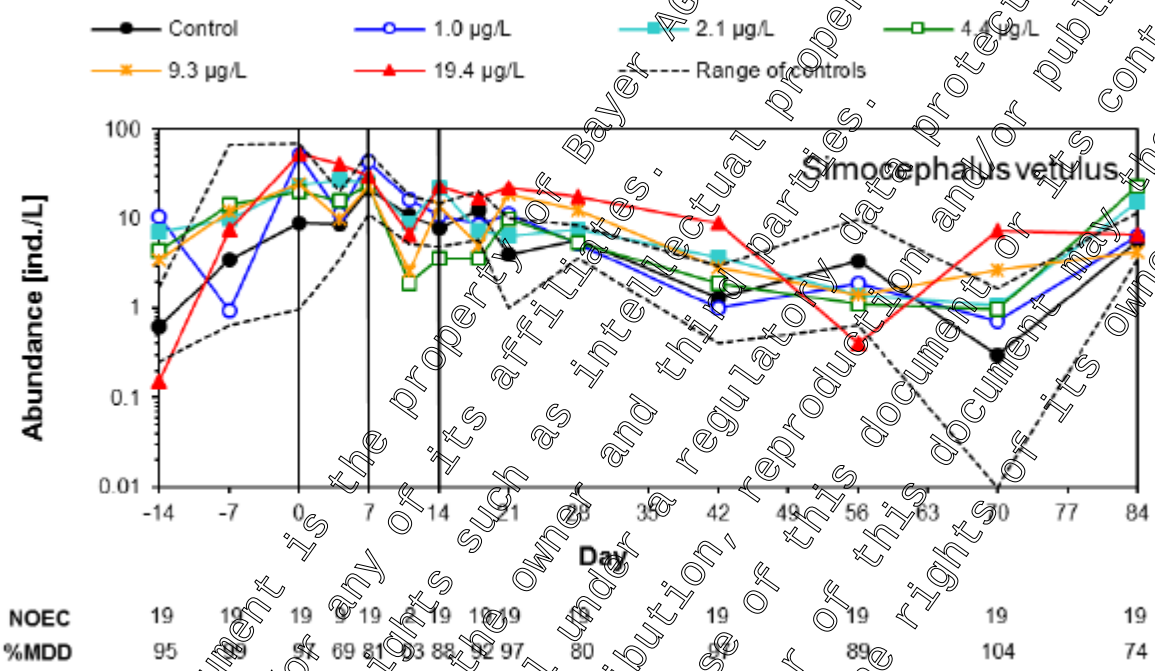
NOEC	19	19	19	19	19	2	19	19	1			19	19	
%MDD	84	91	89	85	83	85			78		90	85	89	

Figure CP 10.2.3/02-8 *Daphnia longispina*: Geometric means per treatment level and range of controls



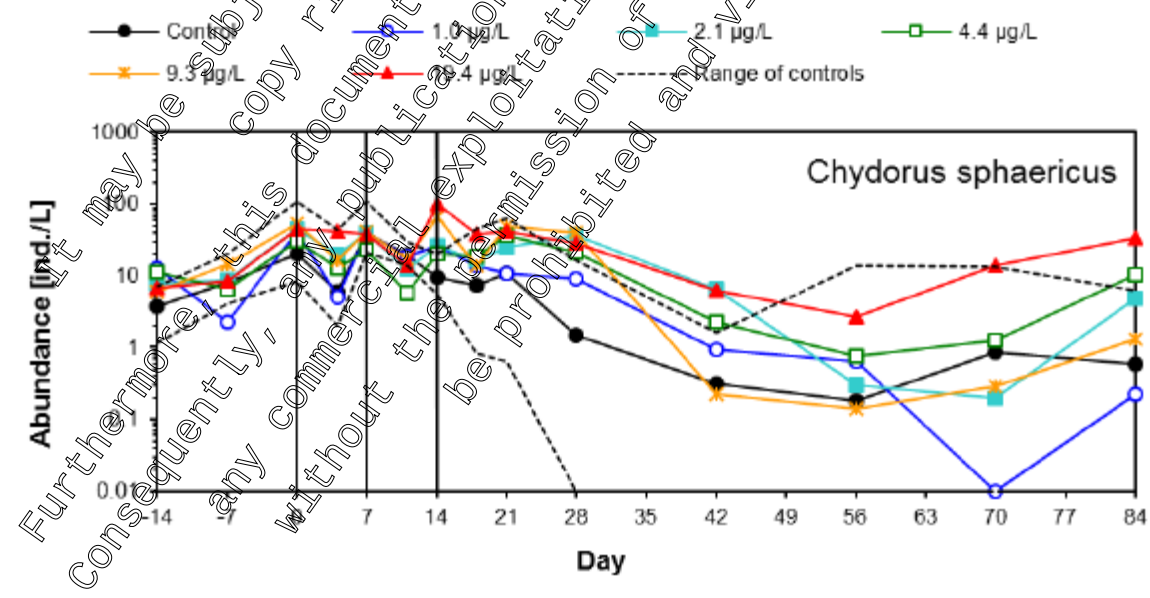
NOEC	19	19	19	19	19	19	19	19	19		2	9	19	19
%MDD	121	103	100	101	100	101	98	99	93	84	84	84	155	167

Figure CP 10.2.3/02-9 ***Simocephalus vetulus*: Geometric means per treatment level and range of controls**



NOEC	19	19	19	9	19	19	19		19		19		19	19
%MDD	95	99	97	69	81	93	88	92	97	80	91	89	104	74

Figure CP 10.2.3/02-10 ***Chydorus sphaericus*: Geometric means per treatment level and range of controls**



NOEC	19	19	19	19	19	19	4	19	19	19	19	19	19	19
%MDD	85	90	95	93	85	56	80	98	98	101	107	114	103	104

Figure CP 10.2.3/02-11 Sum of Daphnids: Geometric means per treatment level and range of controls

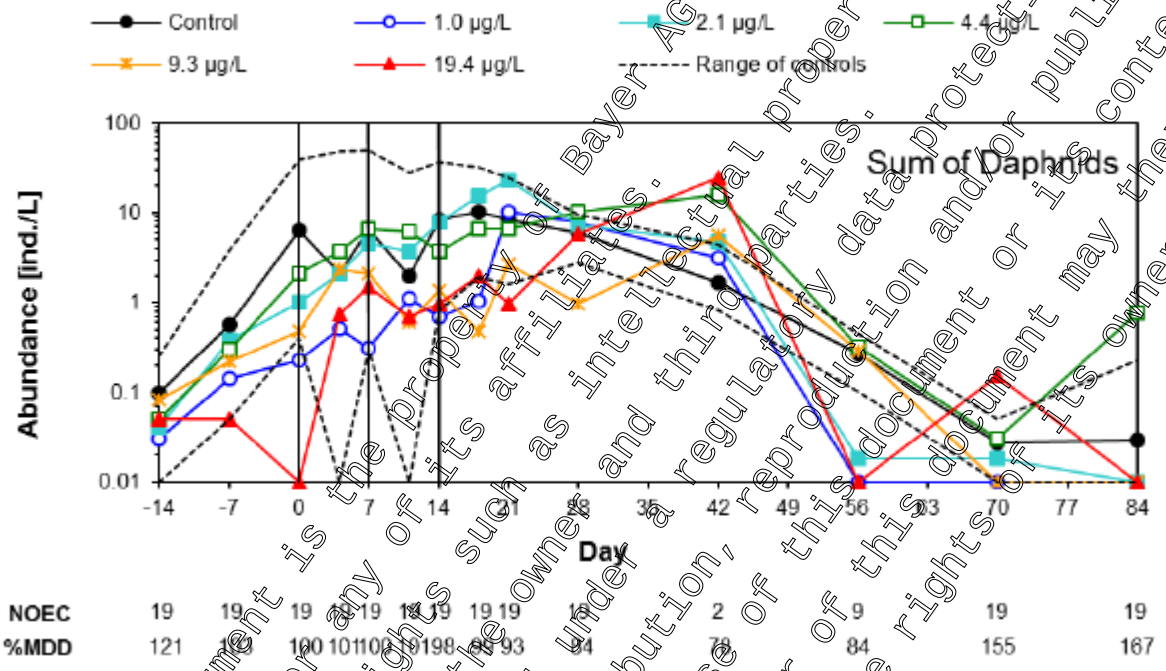


Figure CP 10.2.3/02-12 *Synchaeta* spec.: Geometric means per treatment level and range of controls

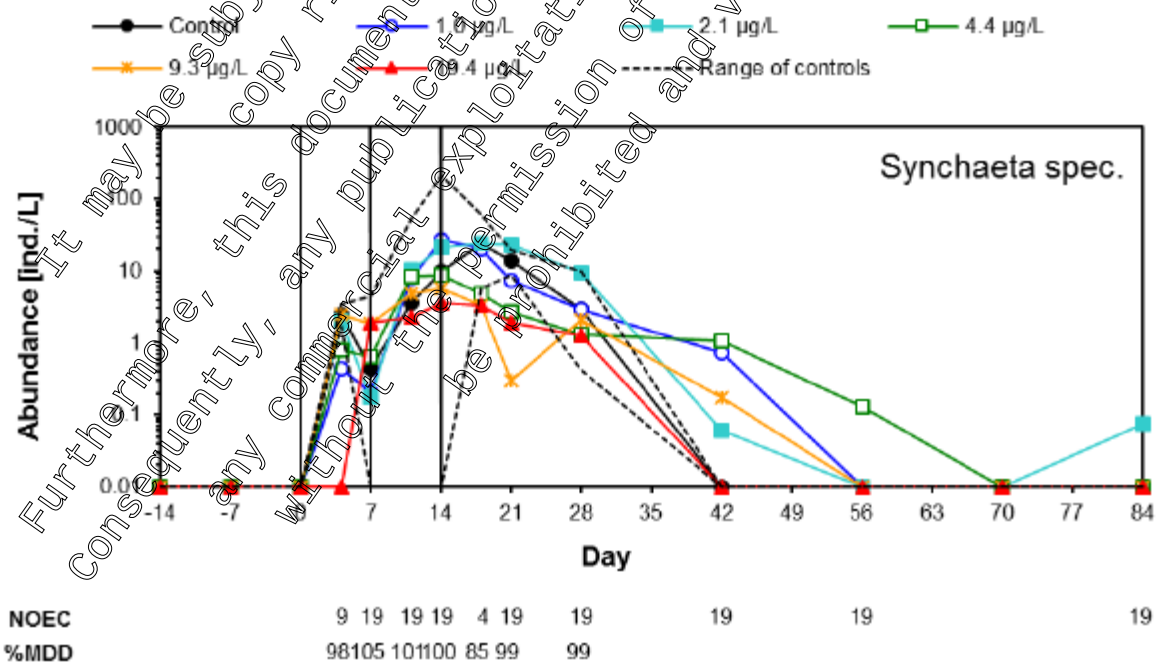
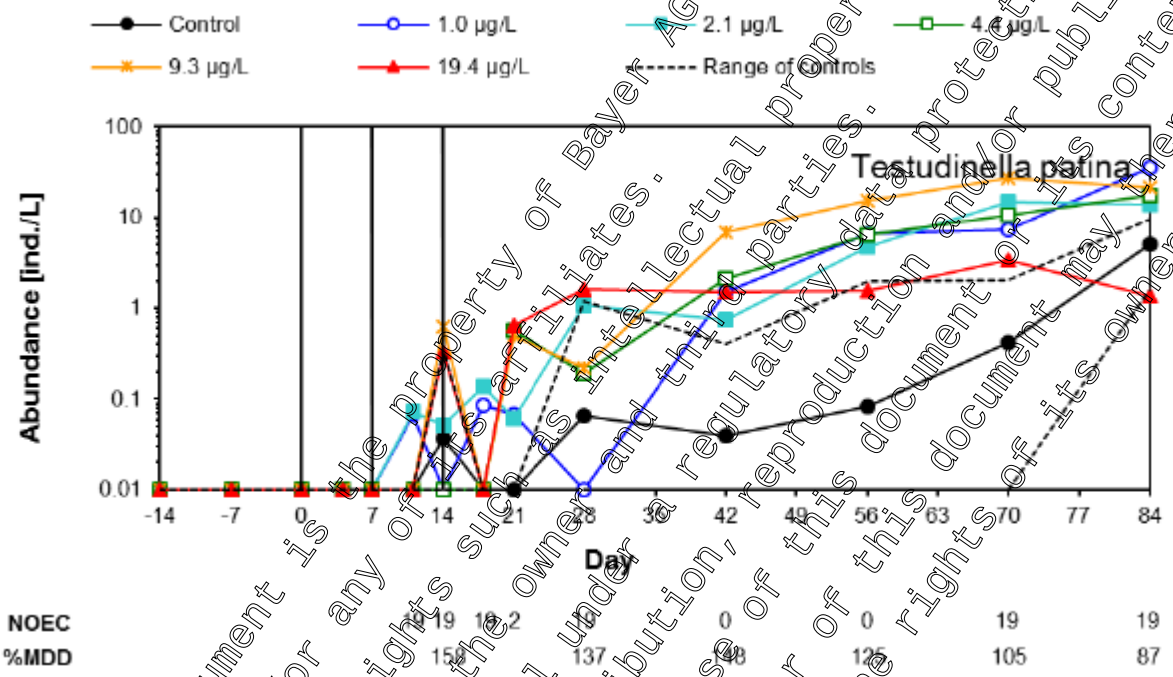


Figure CP 10.2.3/02-13 ***Testudinella patina*: Geometric means per treatment level and range of controls**



Macrozoobenthos

Macrozoobenthos organisms were sampled using artificial substrate samplers (ASS). In the data set, six taxa fulfil the MDD criterion defined by Brock *et al.* (2015): Chironomidae in total, Chironominae, *Chironomini gen spec*, *Chaoborus crystallinus* larvae, leeches (Hirudinea) and Oligochaeta.

Table CP 10.2.3/02-7 % MDDs for the taxa in the macroinvertebrate data set which met the criterion proposed by Brock *et al.* (2015). MDD category 1

Zooplankton	Summary			
	Min	Max	Mean	MDD Cat
Sum of Chironomids	55	262	112	1
Sum of Chironominae	55	171	95	1
Sum of leeches	73	226	107	1
Sum of Oligochaeta	64	138	91	1
Chironomini gen spec.	52	225	108	1
Chaoborus crysallinus larvae	82	148	101	1

MDD cat = category based on MDD evaluation according to Brock et al. (2015).

Furthermore, for 13 taxa, the MDD criterion was not met, but on at least one sampling date after application, a significant difference to the controls was found (MDD category 2 taxa).

Table CP 10.2.3/02-8 % MDDs for the taxa in the macroinvertebrate data set which met the criterion proposed by Brock *et al.* (2015). MDD category 2

Zooplankton	Summary			
	Min	Max	Mean	MDD Cat
Sum of Tanypodinae	71	225	115	2
Stylaria lacustris	72	122	97	2
Herpobdella octoculata	71	262	131	2
Gastropoda non det.	n.c.	n.c.		2
Gyraulus albus	97	186	136	2
Musculium lacustre	94	218	167	2
Pisidium spec	117	225	186	2
Caenis spec	65	112	89	2
Tanypodinae gen spec	71	225	115	2
Tanytarsini gen spec.	186	192	189	2
Culex spec	93	93	93	2
Hydrophilidae gen spec	92	92	92	2
Zygoptera Gen spec	n.c.	n.c.		2
Dugesia gonocephala	84	272	153	2

MDD cat = category based on MDD evaluation according to Brock et al. (2015)

No consistent significant differences to controls over at least two consecutive sampling dates were found for the taxa in the MASS data set; only on isolated sampling occasions NOECs <19.4 µg/L were detected.

Sums of Chironomidae as well as Chironominae were significantly reduced in the highest treatment on day 28 while similar reductions were observed on day 21 however without statistical significance. Later, the abundance in the mesocosm treated with 19.4 µg/L was close to control level again. The deviation of the mean abundance in the 2.1 µg/L mesocosms from controls on day 28 was not considered treatment related, because no concentration response relation was given. Thus, only the slight temporary lower abundances at 19.4 µg/L were considered a class 2 effect. For Chironomini, a NOEC of 1 µg/L was calculated but since the mean abundances at 4.4 and 9.3 µg/L were very close to the control and while the mean abundance at 2.1 µg/L showed the lowest abundance, also here only the slightly reduced abundance at 19.4 µg/L was considered to indicate a slight treatment effect.

Table CP 10.2.3/02-9 NOECs [µg/L] and related % MDDs (in brackets) for the taxa in the macrozoobenthos data set

Macrozoobenthos		Days after application										
MDD cat	Taxa / day	-14	-7	0	7	14	21	28	42	56	70	84
1	Sum of Chironomids ASS	≥19.4 (57)	≥19.4 (82)	≥19.4 (80)	≥19.4 (60)	≥19.4 (64)	≥19.4 (85)	9.3-(55)	≥19.4 (93)	≥19.4 (115)	≥19.4 (262)	≥19.4 (161)

Macrozoobenthos		Days after application										
MDD cat	Taxa / day	-14	-7	0	7	14	21	28	42	56	70	84
1	Sum of Chironominae ASS	1-(43)	≥19.4 (85)	≥19.4 (81)	≥19.4 (61)	≥19.4 (66)	≥19.4 (86)	9.3-(55)	≥19.4 (94)	≥19.4 (171)	≥19.4 (n.c.)	≥19.4 (133)
1	Sum of Leeches ASS	≥19.4 (109)	≥19.4 (145)	≥19.4 (128)	≥19.4 (226)	9.3+ (101)	≥19.4 (83)	<1+ (119)	≥19.4 (85)	≥19.4 (179)	≥19.4 (92)	9.3+ (73)
1	Sum of Oligochaeta ASS	≥19.4 (86)	≥19.4 (58)	≥19.4 (61)	≥19.4 (68)	≥19.4 (88)	≥19.4 (80)	≥19.4 (74)	≥19.4 (138)	≥19.4 (114)	<1-(64)	≥19.4 (100)
1	Chironomini gen spec.	1-(46)	≥19.4 (85)	≥19.4 (81)	≥19.4 (62)	≥19.4 (68)	≥19.4 (87)	1-(52)	≥19.4 (92)	≥19.4 (171)	≥19.4 (n.c.)	≥19.4 (225)
1	Chaoborus crystallinus larvae	≥19.4 (165)	≥19.4 (134)	≥19.4 (210)	4.4+ (148)	≥19.4 (90)	≥19.4 (101)	≥19.4 (93)	≥19.4 (83)	≥19.4 (89)	≥19.4 (90)	≥19.4 (112)
2	Sum of Tanypodinae ASS	≥19.4 (97)	≥19.4 (195)	≥19.4 (89)	≥19.4 (98)	≥19.4 (102)	9.3-(71)	≥19.4 (79)	≥19.4 (118)	≥19.4 (114)	≥19.4 (225)	≥19.4 (n.c.)
2	Stylaria lacustris	≥19.4 (88)	≥19.4 (61)	≥19.4 (63)	≥19.4 (72)	≥19.4 (93)	≥19.4 (108)	1-(n.c.)	≥19.4 (n.c.)	≥19.4 (122)	≥19.4 (81)	≥19.4 (107)
2	Herpobdella octoculata	≥19.4 (106)	≥19.4 (140)	≥19.4 (127)	≥19.4 (262)	≥19.4 (223)	≥19.4 (100)	<1+ (129)	≥19.4 (71)	≥19.4 (79)	≥19.4 (97)	≥19.4 (87)
2	Gastropoda non det.		≥19.4 (n.c.)									9.3+ (n.c.)
2	Gyraulus albus	≥19.4 (169)	≥19.4 (246)	9.3+ (n.c.)	9.3+ (138)	≥19.4 (97)	≥19.4 (430)	≥19.4 (186)	≥19.4 (169)	≥19.4 (125)	≥19.4 (113)	≥19.4 (131)
2	Musculium lacustre		≥19.4 (225)	≥19.4 (n.c.)	≥19.4 (n.c.)	≥19.4 (n.c.)	≥19.4 (165)	≥19.4 (218)	≥19.4 (146)	≥19.4 (183)	≥19.4 (94)	≥19.4 (195)
2	Pisidium spec	≥19.4 (133)	≥19.4 (128)	≥19.4 (n.c.)	≥19.4 (n.c.)	≥19.4 (n.c.)	≥19.4 (197)	≥19.4 (225)	≥19.4 (117)	≥19.4 (203)	≥19.4 (n.c.)	≥19.4 (n.c.)
2	Caenis spec				≥19.4 (n.c.)				≥19.4 (n.c.)	≥19.4 (n.c.)	≥19.4 (112)	<1-(65)
2	Tanypodinae gen spec	≥19.4 (97)	≥19.4 (193)	≥19.4 (89)	≥19.4 (98)	≥19.4 (102)	9.3-(71)	≥19.4 (79)	≥19.4 (118)	≥19.4 (114)	≥19.4 (225)	≥19.4 (n.c.)
2	Tanytarsini gen spec.	≥19.4 (99)	≥19.4 (143)	≥19.4 (160)	≥19.4 (186)	4.4+ (n.c.)	≥19.4 (n.c.)	≥19.4 (n.c.)	≥19.4 (n.c.)			≥19.4 (192)
2	Culex spec							<1-(93)				
2	Hydrophilidae gen spec		≥19.4 (n.c.)							<1-(92)		
2	Zygoptera Gen spec							9.3+ (n.c.)	4.4+ (n.c.)	≥19.4 (n.c.)		
2	Dugesia gonocephala		≥19.4 (142)	9.3+ (n.c.)		≥19.4 (n.c.)	9.3+ (n.c.)	≥19.4 (272)	≥19.4 (n.c.)	≥19.4 (134)	≥19.4 (121)	1-(84)

Signs indicate the direction of a significant effect and colours indicate the different NOECs. Blank fields: taxon not present. n.c.: MDD could not be calculated because of absence in the controls. Empty cells indicate absence in all samples of that day. Cat = MDD category according Brock et al. (2015).

Oligochaeta were clearly not affected until day 42. The deviations form controls later and the single NOEC of < 1 µg/L are assumed to be not caused by the treatment but by chance due to the low numbers in general. Thus, effect class 1 was used up to 19.4 µg/L.

Hirudinea (leeches) and Chaoborus crystallinus showed significantly higher abundances than in controls on single samplings. The NOEC of < 1 µg/L for leeches on day 28 is caused by a lower abundance in the control on that single date rather than by an increase in abundance in all the treated mesocosms. Therefore, this is not considered as a promotion in all treated mesocosms. However, due to a trend of higher abundances in the mesocosm treated with 19.4 µg/L over several sampling dates, a potential

slight promotion is considered for 19.4 µg/L. Because significantly higher abundance was also found at the last sampling day, class 2+/4A+ was assumed.

Numbers of Chaoborus crystallinus in the ASS were relatively small (< 5 / sample before day 14) and therefore the calculated NOEC of 4 µg/L on day 7 was not considered to indicate an effect (class 1).

Figure CP 10.2.3/02-14 Sum of Chironomids: Geometric means per treatment level and range of controls

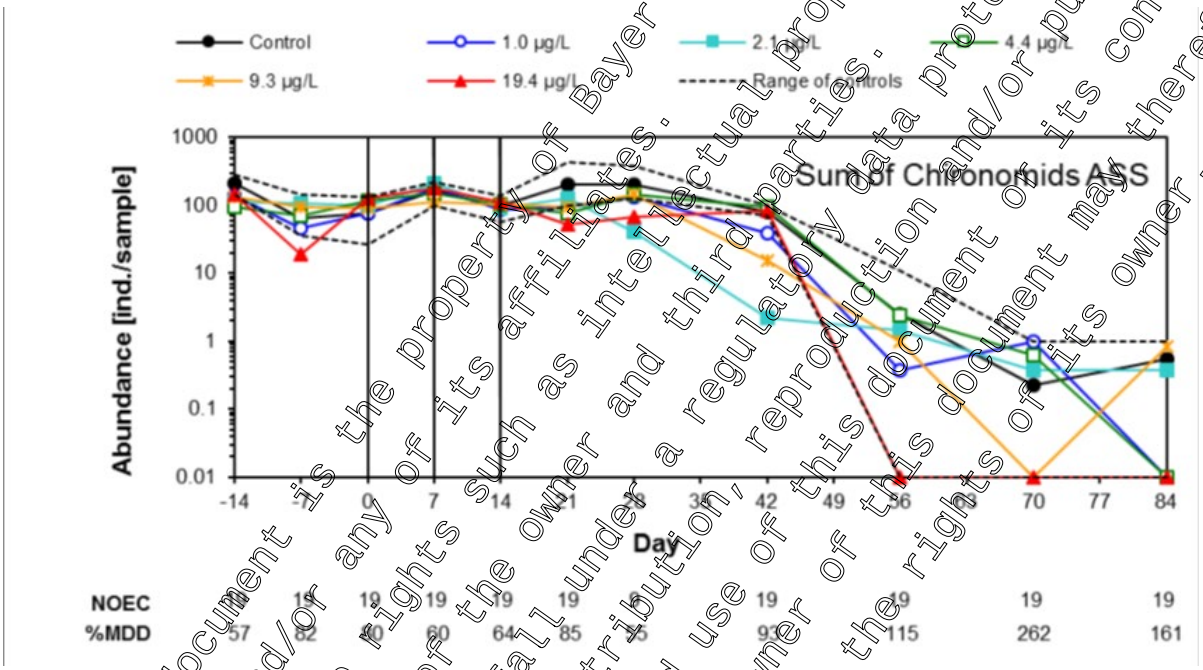


Figure CP 10.2.3/02-15 Sum of Chironominae: Geometric means per treatment level and range of controls

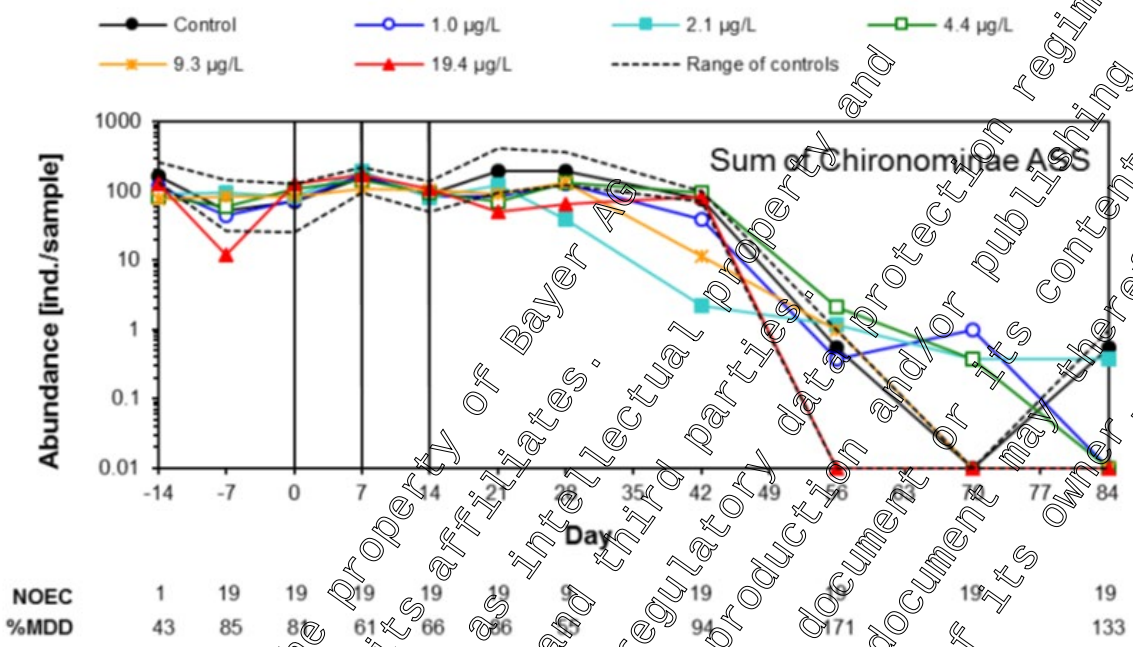


Figure CP 10.2.3/02-16 Chironomini gen spec.: Geometric means per treatment level and range of controls

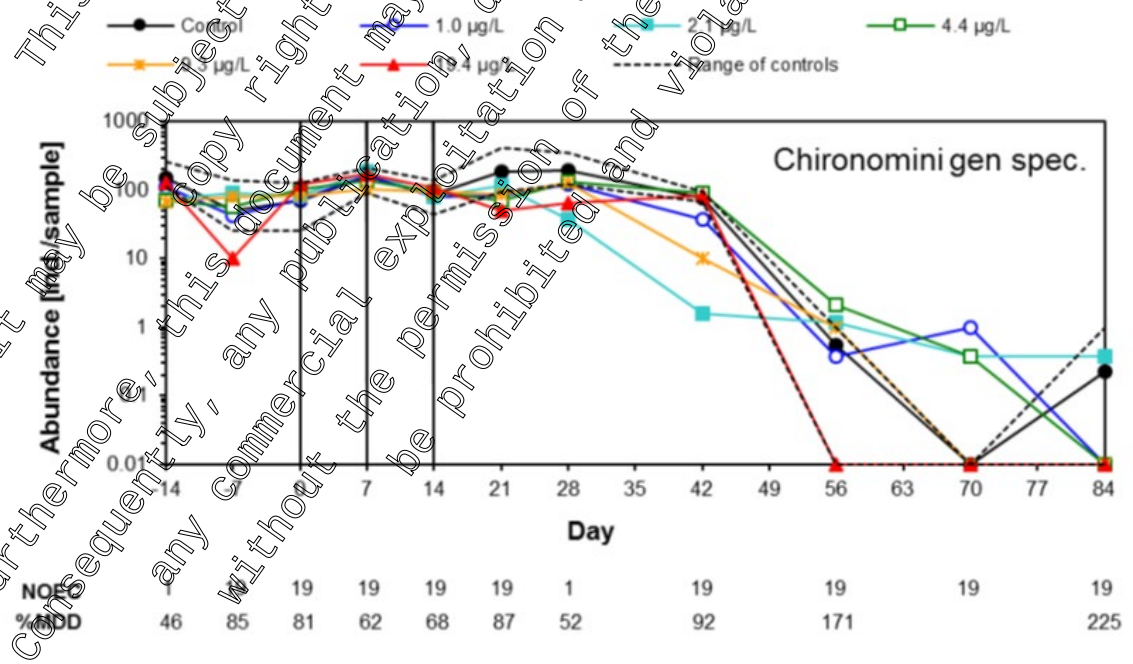


Figure CP 10.2.3/02-17 Sum of Oligochaeta: Geometric means per treatment level and range of controls

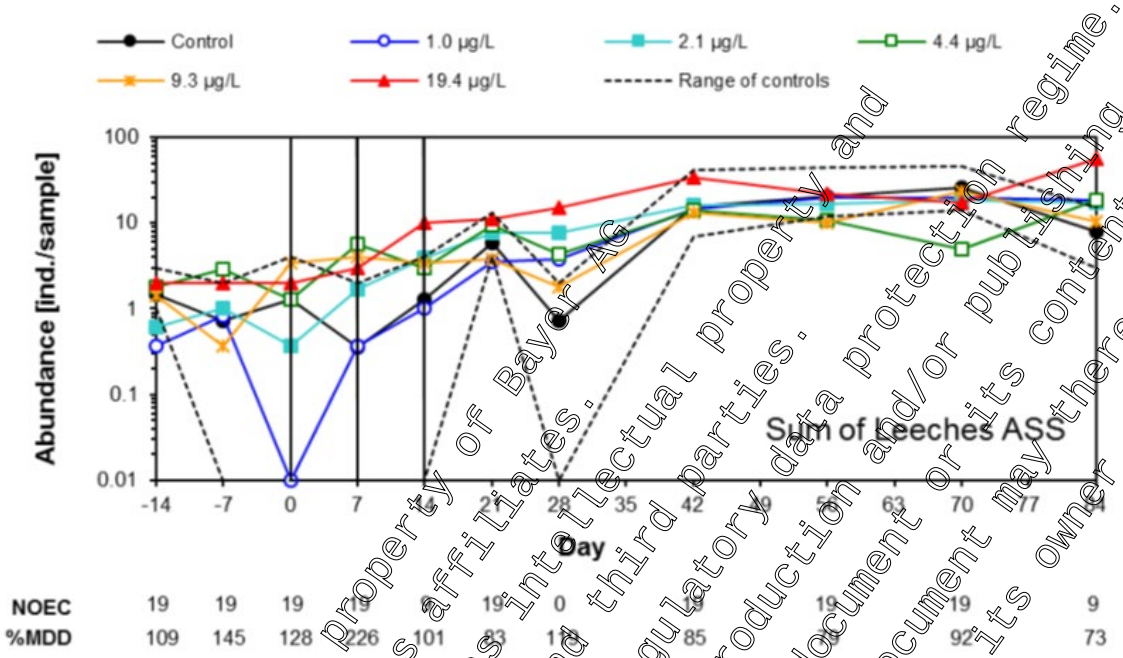
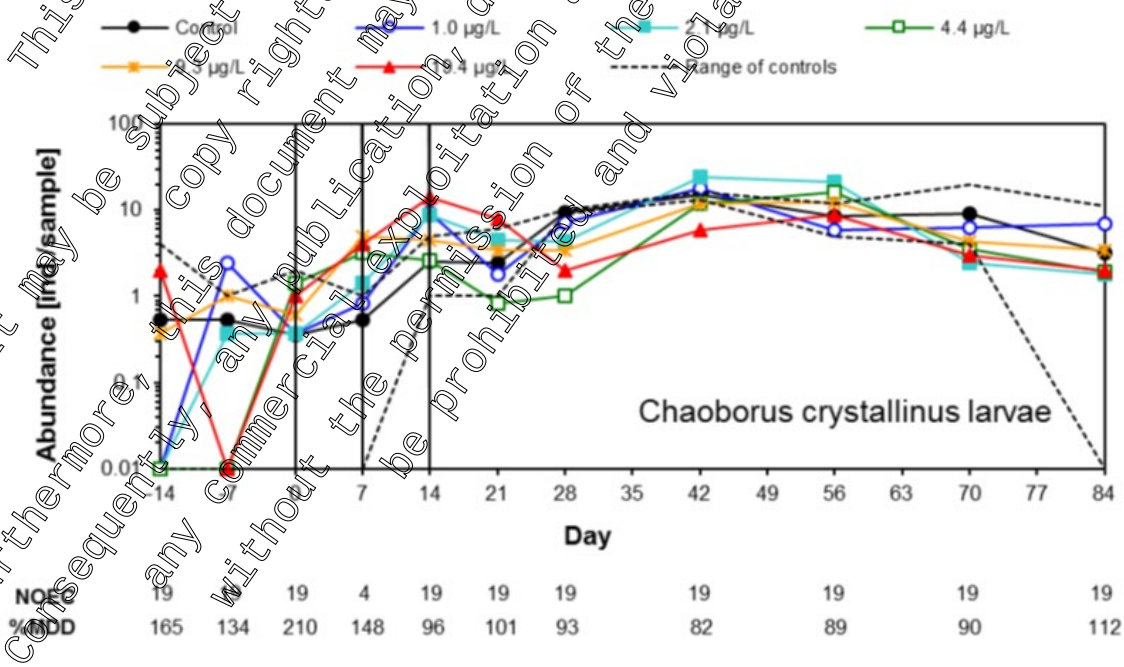


Figure CP 10.2.3/02-18 Sum of Hirudinea (leeches): Geometric means per treatment level and range of controls



Most of the taxa in MDD category 2 showed a short promotion on single samplings without a difference to the control at the end of the study (e.g. *Herpobdella octoculata*, *Gyraulus albus, Pisidium spec and Tanytarsini gen spec.*). Others were extremely rare (*e.g. Culex spec.*, Hydrophilidae). For two taxa (*Caenis spec* and *Dugesia gonocephala*) a significant decrease with a NOEC of 1.0 µg/L was observed on the last sampling on day 84. However, no clear concentration response could be detected and the taxa were found only on isolated samplings. As also these statistical differences are not characterized by a clear concentration-response, these taxa were not considered for effect classification.

Phytoplankton

The effects on phytoplankton were evaluated by means of identification and enumerating of the cells using a reversed microscope and by means of measurements of chlorophyll a content.

Phytoplankton counts

Algae of seven classes were identified in the outdoor mesocosm study: Cryptophyceae, Diatomeae, Chlorophyceae, Chrysophyceae, Conjugatophyceae, Cyanobacteria and Euglenophyceae. For four of them plus the total sum of algae, and seven of the differentiated taxa, the MDDs were sufficiently low to allow an evaluation of direct effects. For several other taxa, significant differences were detected despite the MDDs did not meet the criterion defined by Brock *et al.* (2015) (MDD category 2 taxa).

Table CP 10.2.3/02-10 % MDDs for the taxa in the phytoplankton counts which met the criterion proposed by Brock *et al.* (2015). MDD category 1

Phytoplankton	Summary			
	Min	Max	Mean	MDD Cat
Sum algae	34	81	60	1
Sum Chlorophyceae	42	95	75	1
Sum Chlorophyceae	67	95	79	1
Sum Diatoms	48	101	84	1
Sum Cyanobacteria	75	101	90	1
Pseudoanabaena spec.	75	101	90	1
Chlamydomonas spec.	42	115	94	1
coccoid Chlorophyceae	59	182	101	1
Chroomonas spec.	67	136	95	1
Cryptomonas spec. 20-30 µm	45	93	76	1
Achnanthes spec.	75	99	90	1
Pennales 20-30 µm	72	230	117	1

MDD cat = category based on MDD evaluation according to Brock et al. (2015).

Table CP 10.2.3/02-11 % MDDs for the taxa in the phytoplankton data set which met the criterion proposed by Brock *et al.* (2015). MDD category 2

Zooplankton	Summary			
	Min	Max	Mean	MDD Cat
Sum_Euglenophyceae	111	208	158	2
Merismopedia spec.	n.c.	n.c.		2

Zooplankton	Summary			
	Min	Max	Mean	MDD Cat
Phacus pleuronectis	92	92	92	2
Euglena spec.	119	221	170	2
Trachelomonas spec.	119	249	178	2
Scenedesmus cf Dimorphus	104	136	120	2
Characium spec.	84	223	134	2
Ankyra judayi	90	189	125	2
Closterium cf leibleinii	146	248	190	2
Cocconeis spec.	102	269	178	2
Synedra ulna	156	161	159	2
Pennales 30-40 µm	61	172	116	2
Pennales 70-80 µm	198	203	186	2

MDD cat = category based on MDD evaluation according to Brock et al. (2015).

For the sum of algae, significantly reduced abundances in comparison to controls were detected on four consecutive samplings (day 4 – day 14), with NOEC of 1 µg/L on day 11, 2.1 µg/L on day 7 and 4.4 µg/L on day 4 and 14. Upon the last application, on day 14, the sum of algae recovered quickly and no significant effects were detected (effect class 3A for 4.4 µg/L and higher test concentrations; effect class 2 for 2.1 µg/L).

Cryptophyceae were the most abundant and one of the most affected groups. Prolonged effects (day 4 – day 28) were detected on total Cryptophyceae, in particular on Cryptomonas spec. (20-30 µm) and Chroomonas spec. Until day 28, the Cryptophyceae were affected in the two highest treatments of 9.3 and 19.4 µg/L (effect class 3A), while the lower abundances at 4.4 µg/L were only significant on day18 (class 2). However, for Cryptomonas spec. (20-30 µm) significantly lower abundances were detected already in the mesocosms treated with 4.4 µg/L (day 11 – day 21) with recovery until day 56 (effect class 3A). Chroomonas spec. was less sensitive, with pronounced short-term effects at 9.3 and 19.4 µg/L (effect class 3A).

For total Chlorophyceae, significantly reduced abundances were detected on day 4 at the two highest treatments of 9.3 and 19.4 µg/L and on day 11 in all treatments. However, it seems unlikely that on day 11 all treatment levels had a direct effect since there was clearly no effect after the first and the third application. After the third application, the strongest growth was found in the mesocosm treated with 19.4 µg/L leading to significantly higher abundances over 2 weeks. Thus, this promoting effect of the three applications was considered more relevant for the effect classification (effect class 3A at 19.4 and 2 at 9.3 µg/L). On day 7, a NOEC of 1 µg/L was calculated for the green algae Chlamydomonas spec.. This isolated NOEC is considered not to be treatment related as in the higher treatments, the abundances were in the range of controls. Thus, effect class 1 was used for all treatment levels for this species. For the coccoid Chlorophyceae, a significant reduction was detected on two sampling occasions (day 11 and 21) for the three, respectively two highest test concentrations of 19.4, 9.3 and 4.4 µg/L and was considered to be an effect class 2.

The total Diatoms were not affected except on day 18, when significant differences to the control were detected for the highest test concentration of 19.4 µg/L (effect class 2). For the diatom Achnanthes spec., significantly reduced abundances were detected on day 11, 14, 18, 28 and 42 which was considered as an effect class 2 for 4.4 µg/L and an effect class 3A for 9.3 and 19.4 µg/L. For small Pennales (20-30 µm), significantly higher abundances with a NOEC of 1 µg/L were detected on day 56. Since these

increases showed not concentration-dependent response, it was not considered as an indication of a promoting effect, and class 1 was assumed for all test concentrations.

The cyanobacteria in total and the taxon Pseudoanabaena spec. were not affected during the whole study (effect class 1).

Table CP 10.2.3/02-12 NOECs [µg/L] and related % MDDs (in brackets) for the phytoplankton counts

Macrozoobenthos		Days after application													
MDD cat	Taxa / day	-14	-7	0	4	7	11	14	18	21	28	42	56	70	84
1	Sum algae	≥19.4 (49)	≥19.4 (36)	≥19.4 (39)	4.4- (45)	2.1- (34)	1- (52)	4.4- (50)	9.3- (46)	≥19.4 (78)	≥19.4 (81)	≥19.4 (68)	≥19.4 (79)	≥19.4 (79)	≥19.4 (81)
1	Sum chlorophyceae	≥19.4 (71)	≥19.4 (95)	≥19.4 (80)	4.4- (54)	≥19.4 (71)	1- (52)	≥19.4 (87)	9.3+ (67)	9.3+ (84)	4.4+ (69)	≥19.4 (79)	≥19.4 (88)	≥19.4 (92)	≥19.4 (95)
1	Sum cryptophyceae	≥19.4 (56)	≥19.4 (37)	≥19.4 (37)	4.4- (77)	4.4- (72)	4.4- (82)	4.4- (74)	2.1- (67)	9.3- (70)	4.4- (72)	≥19.4 (68)	≥19.4 (86)	≥19.4 (92)	≥19.4 (84)
1	Sum diatoms	≥19.4 (61)	9.3+ (48)	≥19.4 (76)	≥19.4 (69)	≥19.4 (48)	≥19.4 (81)	≥19.4 (101)	9.3- (86)	≥19.4 (89)	≥19.4 (87)	≥19.4 (95)	≥19.4 (89)	≥19.4 (88)	≥19.4 (96)
1	Sum cyanobacteria	≥19.4 (128)	≥19.4 (100)	≥19.4 (102)	≥19.4 (98)	≥19.4 (81)	≥19.4 (101)	≥19.4 (95)	≥19.4 (99)	≥19.4 (98)	≥19.4 (75)	≥19.4 (82)	≥19.4 (84)	≥19.4 (83)	≥19.4 (91)
1	Pseudoanabaena spec	≥19.4 (128)	≥19.4 (108)	≥19.4 (102)	≥19.4 (98)	≥19.4 (81)	≥19.4 (100)	≥19.4 (95)	≥19.4 (98)	≥19.4 (98)	≥19.4 (75)	≥19.4 (82)	≥19.4 (84)	≥19.4 (83)	≥19.4 (91)
1	Chlamydomonas spec.	≥19.4 (157)	≥19.4 (145)	≥19.4 (70)	≥19.4 (100)	1- (42)	≥19.4 (n.c.)	≥19.4 (102)	≥19.4 (99)	≥19.4 (99)	≥19.4 (78)	≥19.4 (97)	≥19.4 (98)	≥19.4 (115)	≥19.4 (114)
1	coccoid Chlorophyceae	≥19.4 (110)		≥19.4 (95)	≥19.4 (67)	≥19.4 (79)	2.1- (59)	≥19.4 (101)	≥19.4 (95)	4.4- (83)	≥19.4 (117)	≥19.4 (117)	≥19.4 (182)	≥19.4 (99)	≥19.4 (118)
1	Chroomonas spec.	≥19.4 (119)	≥19.4 (77)	≥19.4 (119)	≥19.4 (132)	9.3- (81)	4.4- (93)	9.3- (81)	4.4- (67)	≥19.4 (95)	9.3- (78)	≥19.4 (89)	≥19.4 (136)	≥19.4 (112)	2.1+ (85)
1	Cryptomonas spec. 20-30 µm	≥19.4 (135)	≥19.4 (135)	≥19.4 (67)	4.4- (49)	4.4- (58)	2.1- (81)	2.1- (45)	2.1- (89)	2.1- (86)	4.4- (84)	≥19.4 (82)	≥19.4 (93)	≥19.4 (91)	≥19.4 (91)
1	Achnanthes spec.	≥19.4 (157)	≥19.4 (121)	≥19.4 (92)	≥19.4 (90)	≥19.4 (79)	4.4- (94)	≥19.4 (99)	4.4- (84)	≥19.4 (95)	2.1- (75)	9.3- (91)	≥19.4 (94)	≥19.4 (91)	≥19.4 (91)
1	Pennales 20-30 µm	≥19.4 (81)	≥19.4 (80)	≥19.4 (71)	≥19.4 (89)	≥19.4 (78)	≥19.4 (83)	≥19.4 (230)	≥19.4 (226)	≥19.4 (72)	≥19.4 (87)	≥19.4 (119)	1+ (101)	≥19.4 (98)	≥19.4 (99)
2	Sum Euglenophyceae	≥19.4 (177)		≥19.4 (175)	≥19.4 (208)	≥19.4 (189)		≥19.4 (149)	≥19.4 (173)	≥19.4 (150)	9.3+ (125)	≥19.4 (n.c.)	≥19.4 (155)	≥19.4 (189)	≥19.4 (111)
2	Merismopedia spec.				9.3+ (n.c.)					≥19.4 (n.c.)					
2	Phacus pleuronectis				≥19.4 (n.c.)										<1- (92)
2	Euglena spec.									9.3+ (n.c.)		≥19.4 (n.c.)	≥19.4 (221)	≥19.4 (n.c.)	≥19.4 (119)
2	Trachelomonas spec.	≥19.4 (177)		≥19.4 (175)	≥19.4 (193)	≥19.4 (189)	≥19.4 (119)	≥19.4 (173)	≥19.4 (224)		≥19.4 (n.c.)	≥19.4 (n.c.)	≥19.4 (249)	≥19.4 (175)	≥19.4 (153)
2	Scenedesmus dimorphus	≥19.4 (123)	≥19.4 (45)	≥19.4 (126)	≥19.4 (122)	4.4+ (n.c.)	≥19.4 (130)	≥19.4 (136)	≥19.4 (n.c.)	≥19.4 (125)	≥19.4 (120)	≥19.4 (104)	≥19.4 (117)	≥19.4 (108)	≥19.4 (n.c.)
2	Characium spec.		≥19.4 (n.c.)	≥19.4 (n.c.)	≥19.4 (142)		≥19.4 (113)	≥19.4 (185)	≥19.4 (108)	≥19.4 (105)	4.4+ (84)	≥19.4 (152)	≥19.4 (112)	2.1+ (223)	4.4+ (118)
2	Ankyra judayi	≥19.4 (n.c.)			≥19.4 (n.c.)		≥19.4 (112)	≥19.4 (90)	9.3+ (93)	4.4+ (90)	2.1+ (128)	≥19.4 (n.c.)	≥19.4 (189)		≥19.4 (170)
2	Closterium cf leibleinii			≥19.4 (n.c.)	≥19.4 (n.c.)					≥19.4 (248)	≥19.4 (n.c.)	≥19.4 (146)	4.4+ (n.c.)	4.4+ (204)	4.4+ (162)
2	Cocconeis spec.	≥19.4 (n.c.)	≥19.4 (n.c.)	≥19.4 (n.c.)	≥19.4 (162)		2.1+ (n.c.)	≥19.4 (244)	≥19.4 (n.c.)	≥19.4 (199)	≥19.4 (152)	≥19.4 (179)	≥19.4 (269)	≥19.4 (122)	≥19.4 (102)

Macrozoobenthos		Days after application												
MDD cat	Taxa / day	-14	-7	0	4	7	11	14	18	21	28	42	56	70	84
2	Synedra ulna							9.3+ (156)	≥19.4 (161)	≥19.4 (n.c.)	≥19.4 (n.c.)				
2	Pennales 30-40 µm	≥19.4 (89)		≥19.4 (92)	≥19.4 (61)	≥19.4 (110)	≥19.4 (97)	≥19.4 (161)	≥19.4 (172)	≥19.4 (89)	≥19.4 (115)	≥19.4 (122)	9.3+ (n.c.)	≥19.4 (128)	≥19.4 (103)
2	Pennales 70-80 µm			≥19.4 (167)	≥19.4 (186)	≥19.4 (168)	≥19.4 (203)				9.3+ (n.c.)				

Signs indicate the direction of a significant effect and colours indicate the different NOECs. Blank fields: taxon not present. n.c.: MDD could not be calculated because of absence in the controls. Cat = MDD category according Brock et al. (2015).

Figure CP 10.2.3/02-19 Total phytoplankton: Geometric means per treatment level and range of controls

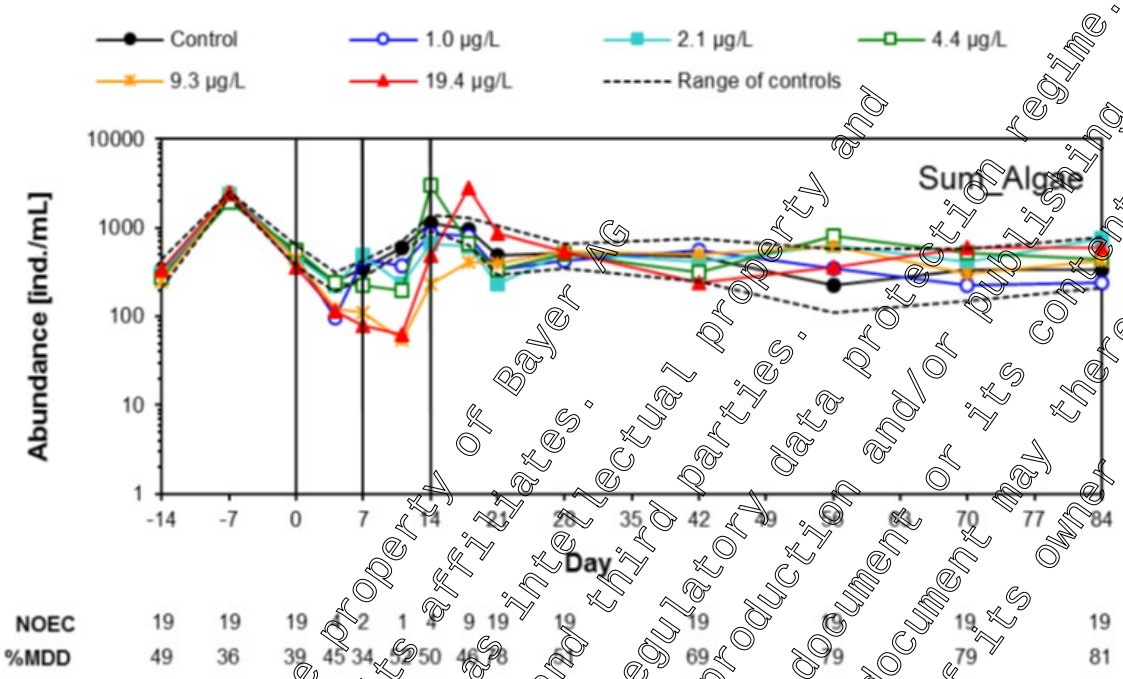


Figure CP 10.2.3/02-20 Sum of Cryptophyceae: Geometric means per treatment level and range of controls

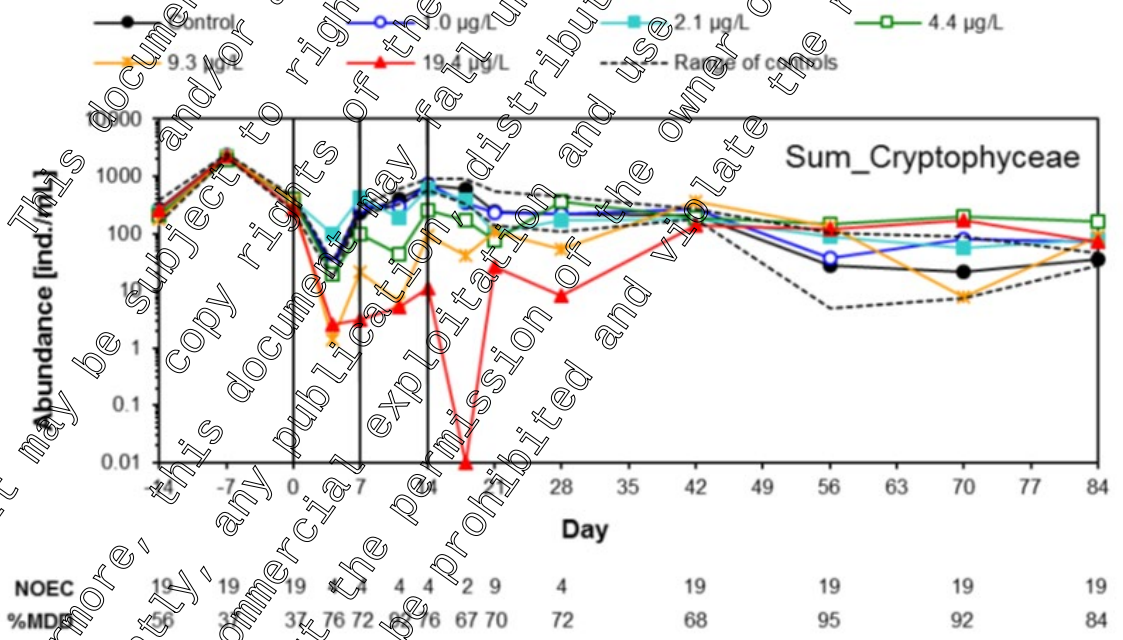


Figure CP 10.2.3/02-21 Cryptomonas spec. 20-30 µm: Geometric means per treatment level and range of controls

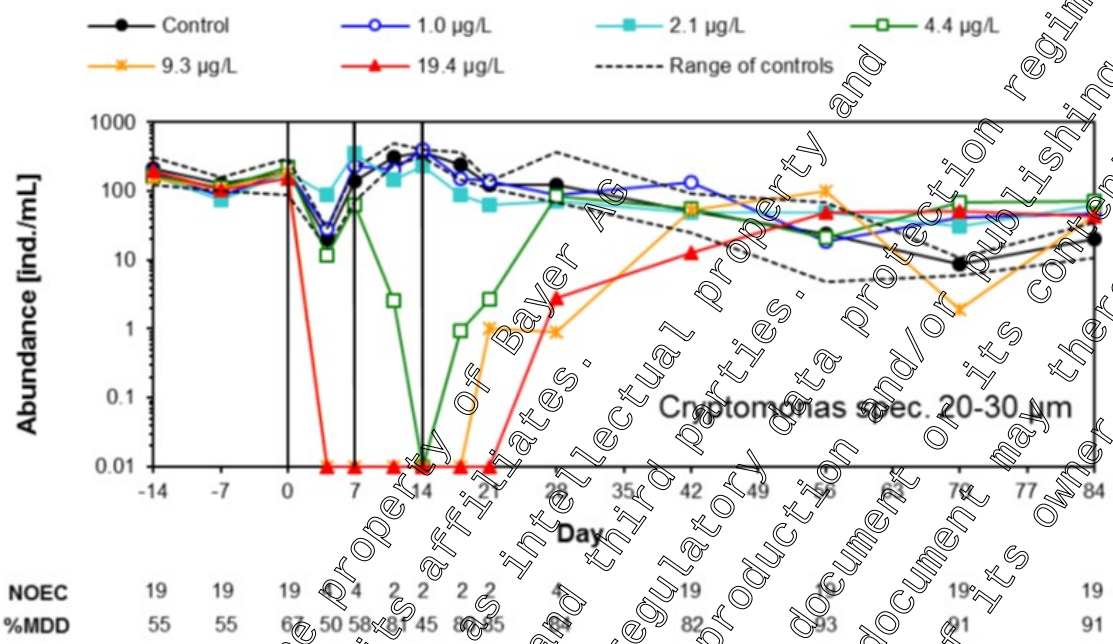


Figure CP 10.2.3/02-22 Chroomonas spec.: Geometric means per treatment level and range of controls

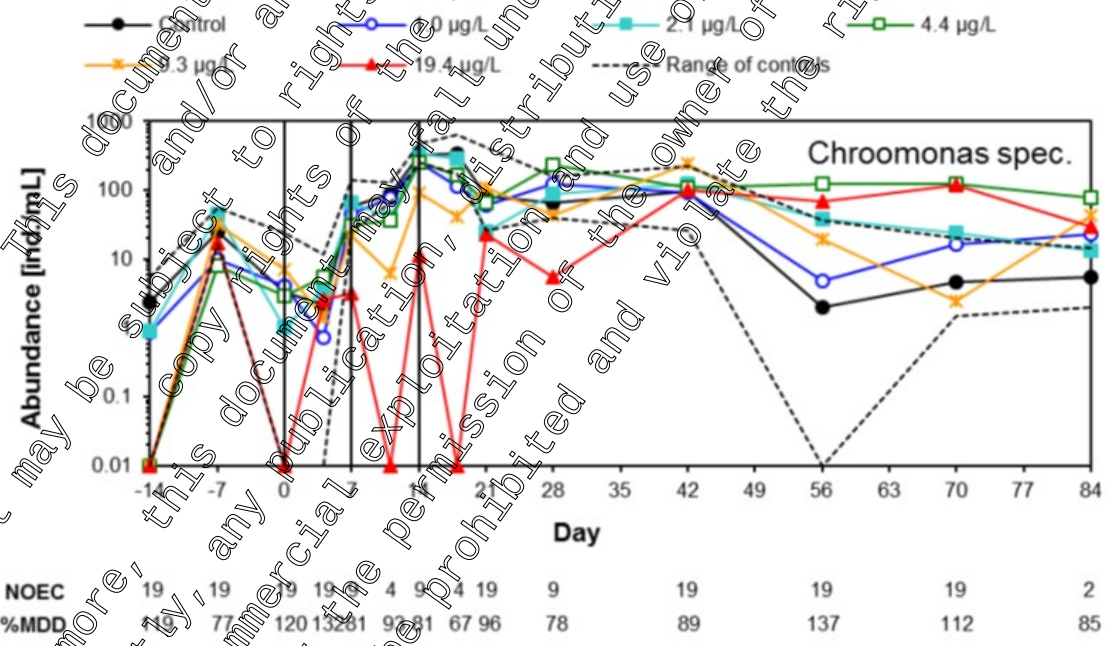
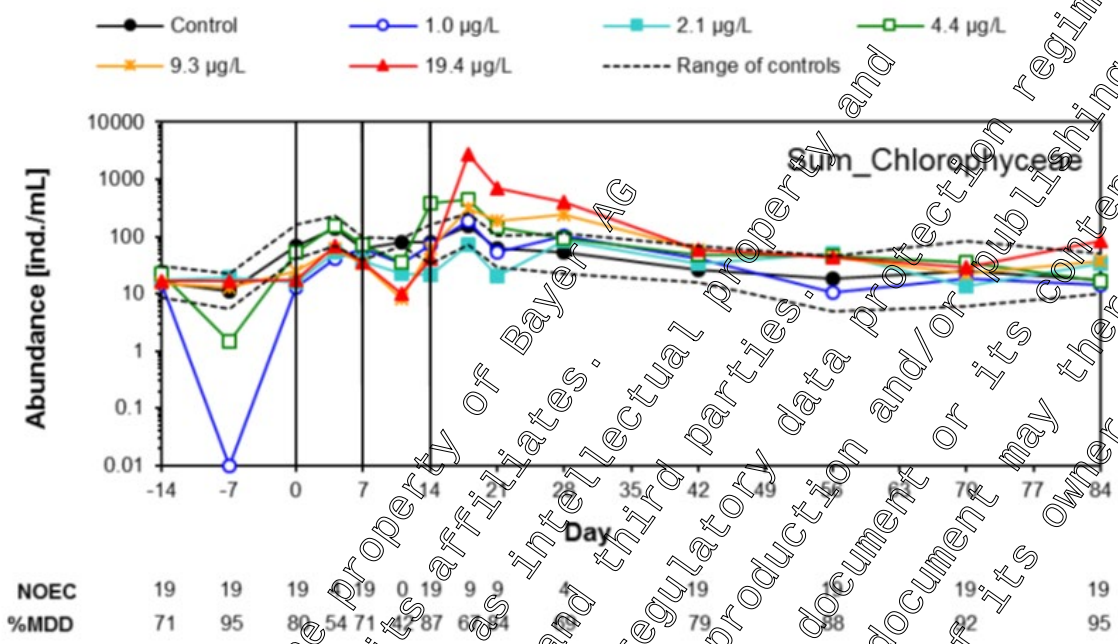
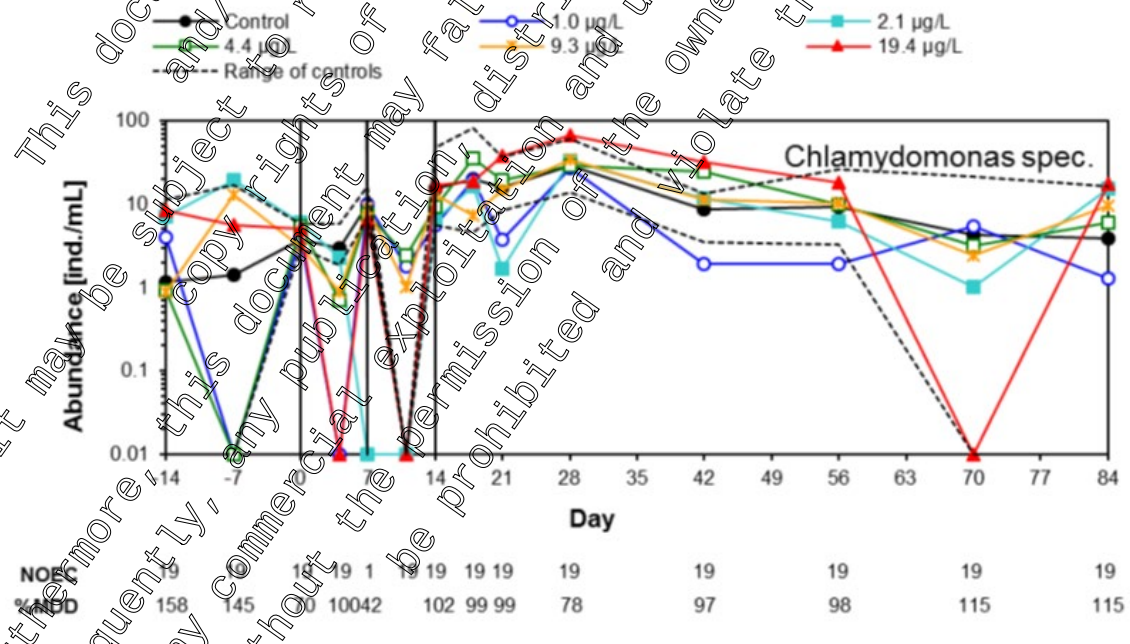


Figure CP 10.2.3/02-23 Sum of Chlorophyceae: Geometric means per treatment level and range of controls



NOEC	19	19	19	19	19	0	19	9	9	4	19	19	19	19
%MDD	71	95	80	54	71	47	87	67	54	79	88	92	95	

Figure CP 10.2.3/02-24 Chlamydomonas spec.: Geometric means per treatment level and range of controls



NOEC	19	19	19	19	1	19	19	19	19	19	19	19	19	19
%MDD	158	145	0	10042		102	99	99	78	97	98	115	115	

Figure CP 10.2.3/02-25 Sum of coccoid Chlorophyceae: Geometric means per treatment level and range of controls

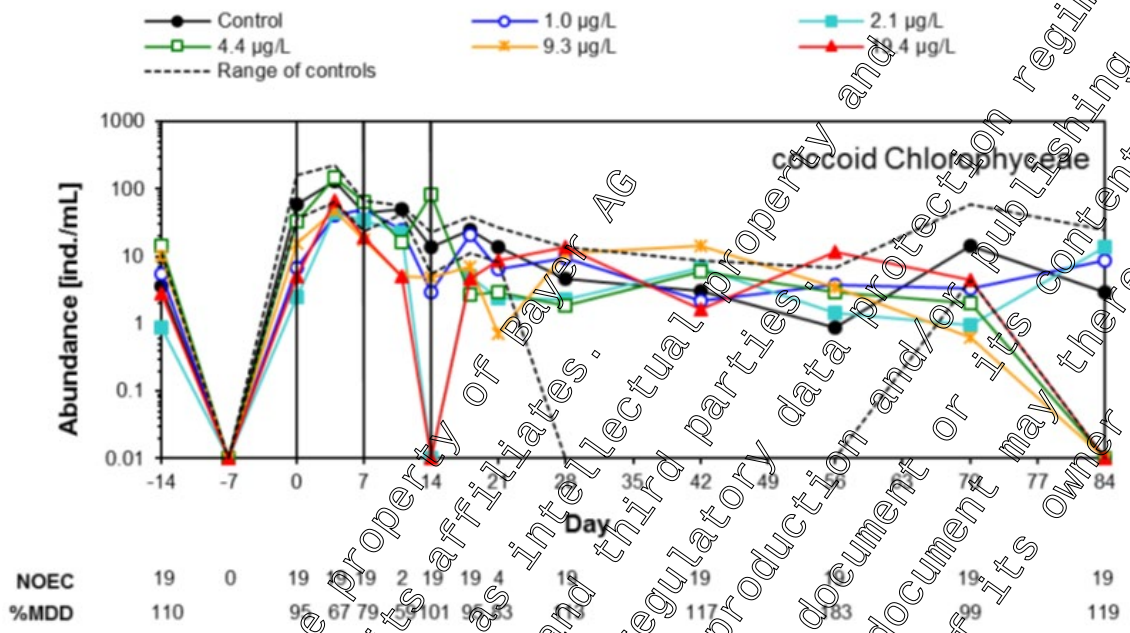


Figure CP 10.2.3/02-26 Sum of Diatoms: Geometric means per treatment level and range of controls

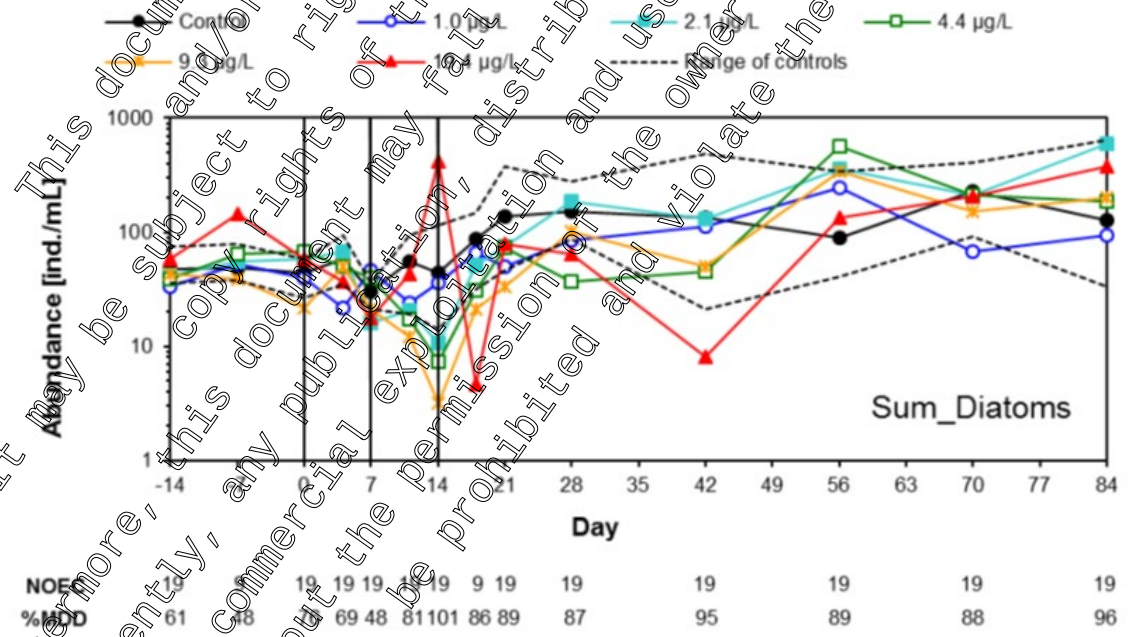


Figure CP 10.2.3/02-27 Achnanthes spec.: Geometric means per treatment level and range of controls

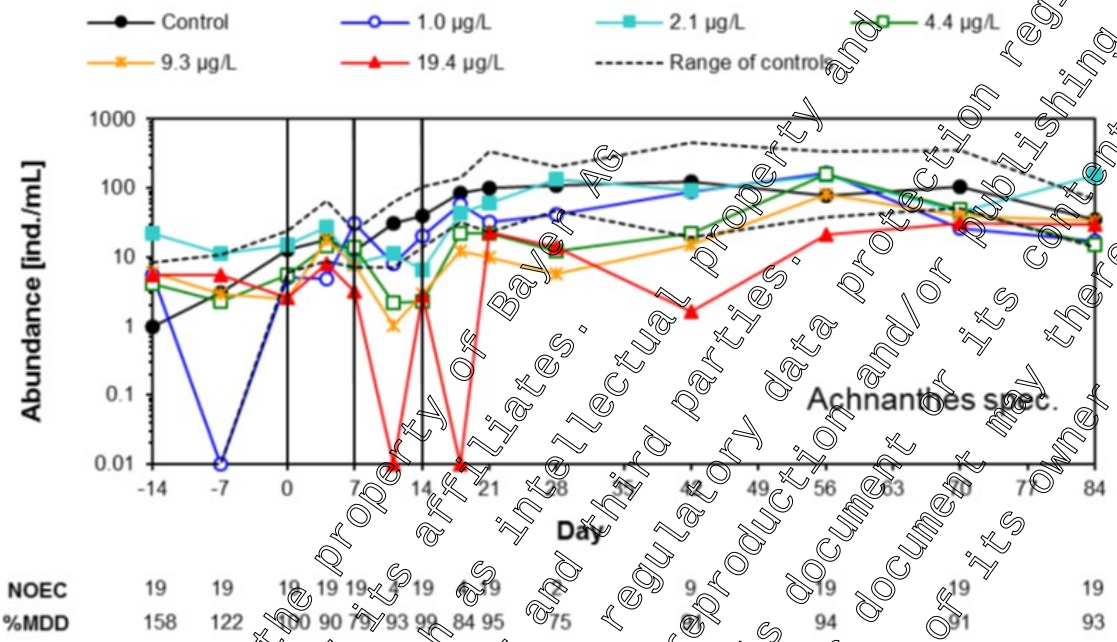


Figure CP 10.2.3/02-28 Pennales (20-30 µm): Geometric means per treatment level and range of controls

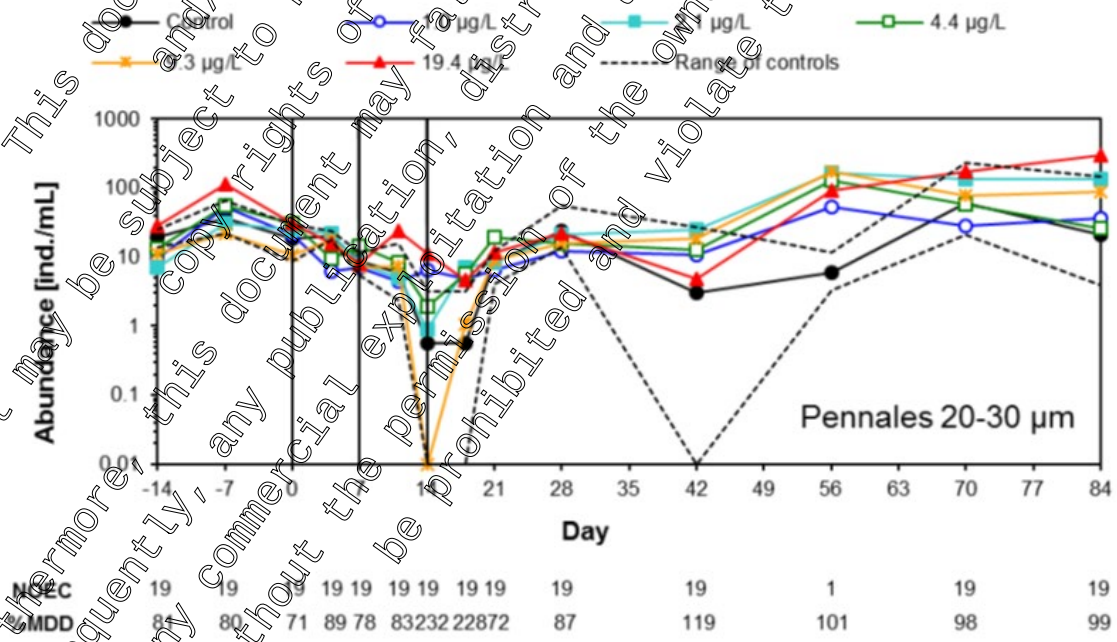


Figure CP 10.2.3/02-29 Cyanobacteria: Geometric means per treatment level and range of controls

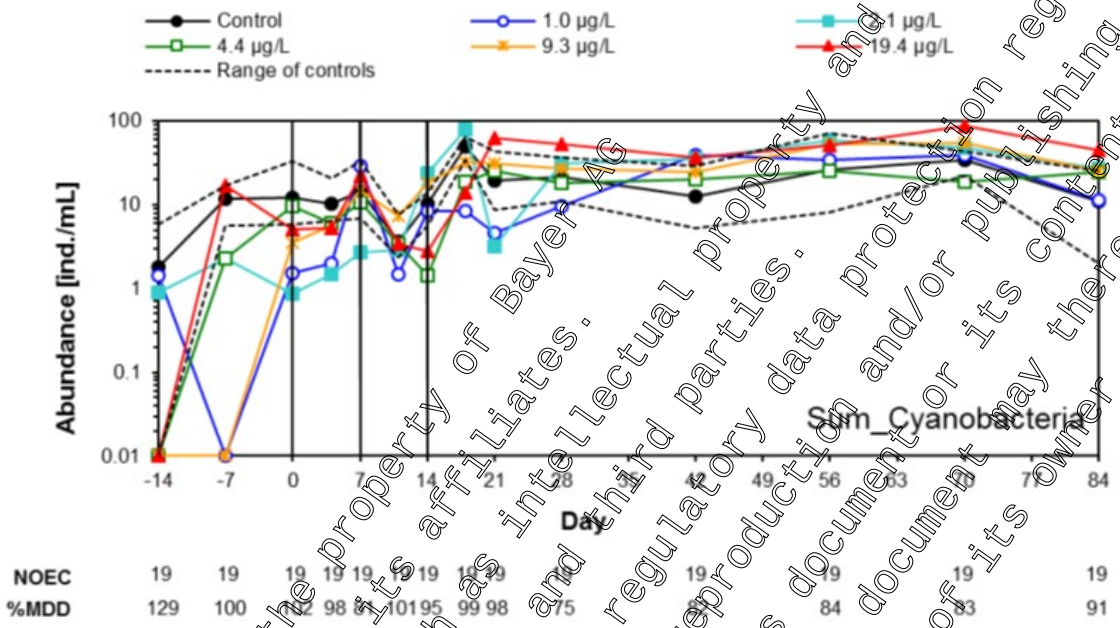
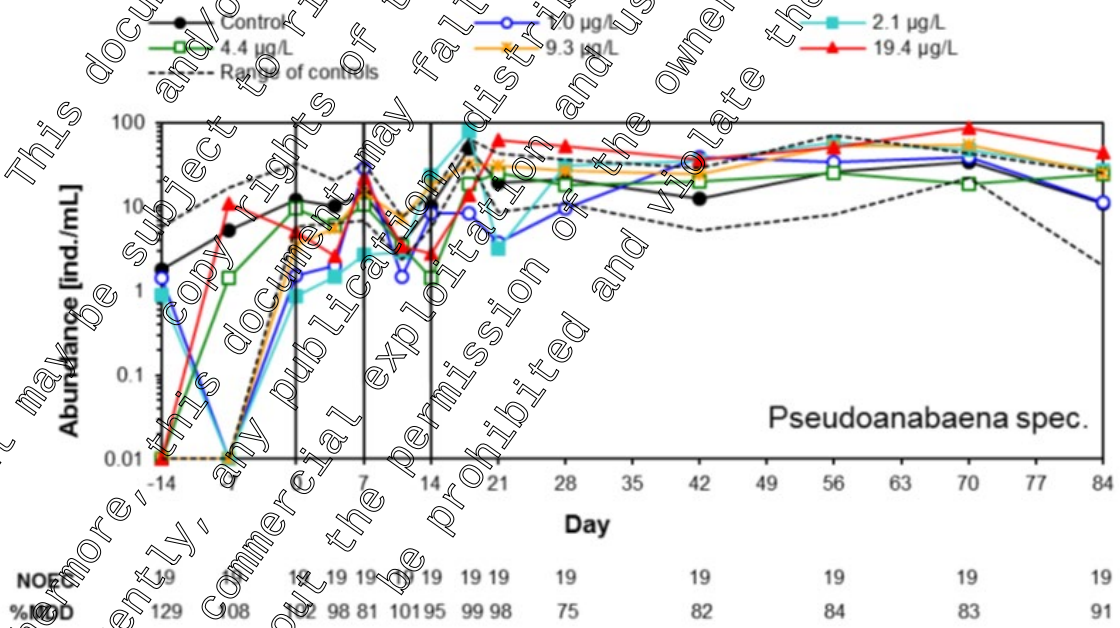


Figure CP 10.2.3/02-30 Pseudoanabaena spec.: Geometric means per treatment level and range of controls



Taxa of MDD category 2 showed increased abundances on single samplings with NOECs of 2.1 µg/L and higher, such as *Merismopedia spec., Euglena spec., Trachelomonas spec. Scenedesmus dimorphus, Cocconeis spec,* Synedra ulna and Pennales. Since this would not affect the final risk assessment, these

taxa were not considered further. However, the green algae *Characium spec., Ankyra judayi, Closterium leibleinii* showed significantly higher abundances than in the controls on 2 or 3 consecutive samplings.

Characium spec. showed significantly higher abundances in the mesocosms treated with 9.3 ad 19.4 µg/L on day 28 (considered as class 2+), and again on day 70 and 84. Since it could not be assessed if the statistical findings at the end of the study indicate a promotion, class 2+/4A+ was considered for this species and the two highest test concentrations.

Higher abundances were also found for Clostridium leibleinii over the last three samplings in the two highest test concentrations. To be conservative, this was interpreted as a potential promotion, effect class 3A+/4A+. However, both species were relatively rare and the higher abundances found at the end of the study did not result in a bloom of algae. Thus, the ecological relevance of the observations is considered to be low.

In contrast, Ankyra judayi showed a significant promotion of abundance on day 18, 21 and 28 with respective NOEC of 9.3, 4.4 and 2.1 µg/L (effect class 3A+ for 9.3 and 19.4 and effect class 2+ for 4.4 µg/L).

Figure CP 10.2.3/02-31 Characium spec.: Geometric means per treatment level and range of controls

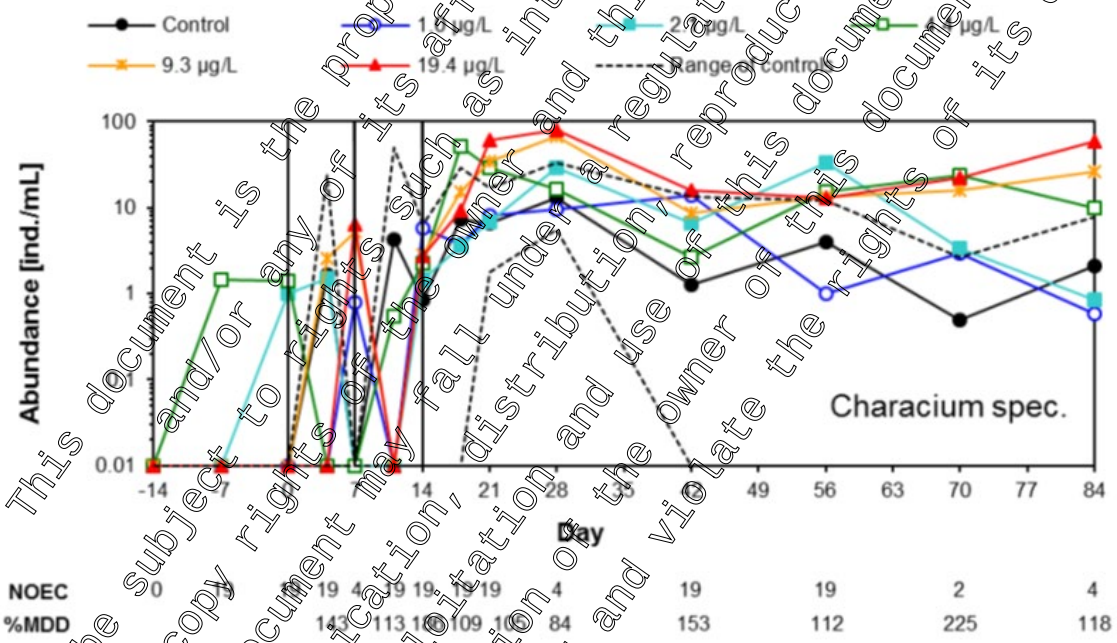


Figure CP 10.2.3/02-32 Closterium cf leibleinii: Geometric means per treatment level and range of controls

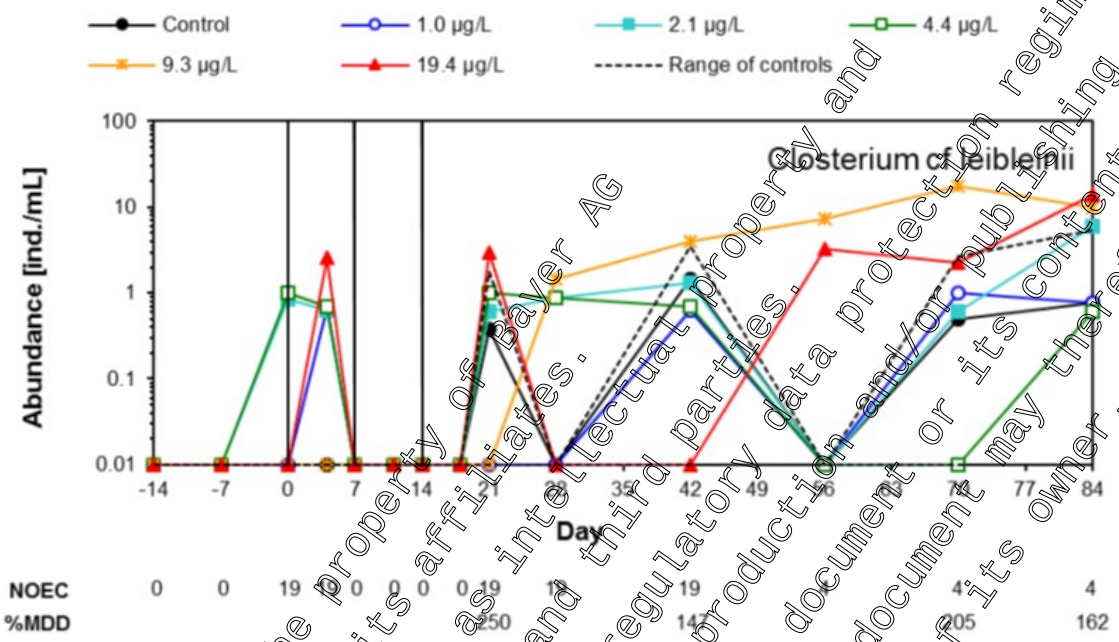
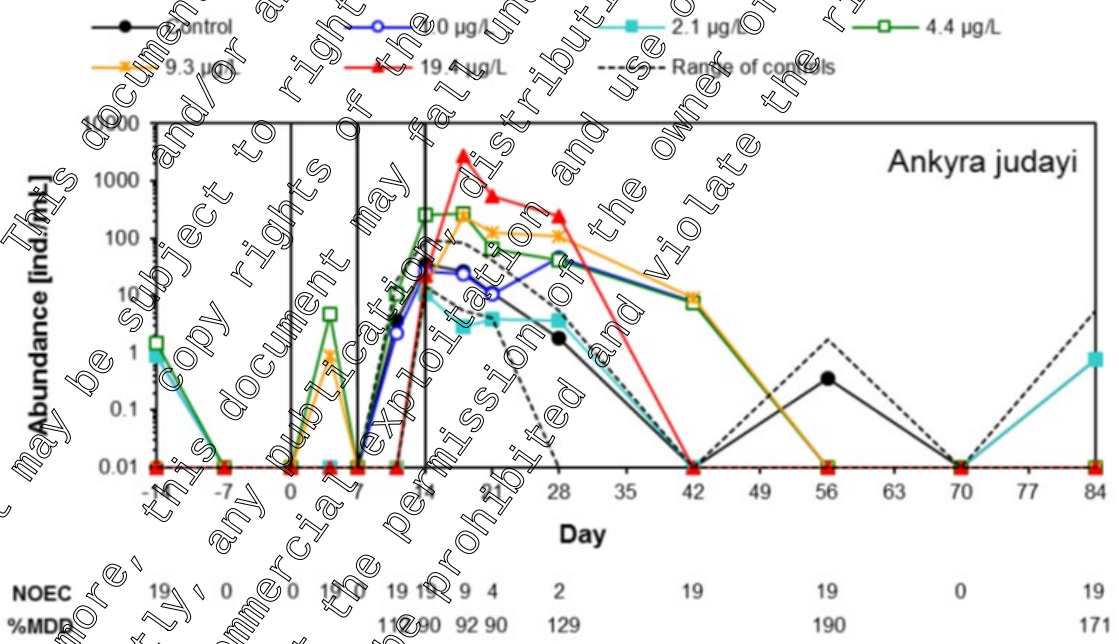


Figure CP 10.2.3/02-33 Ankyra judayi: Geometric means per treatment level and range of controls



Phytoplankton chlorophyll a

The chlorophyll a concentration was measured on several sampling occasions. The calculated MDD demonstrated that small to medium effects could be determined.

Table CP 10.2.3/02-13 % MDDs for phytoplankton chlorophyll a

Phytoplankton	Summary			
	Min	Max	Mean	MDD Cat
Chl-a Phytoplankton	32	108	68	1

MDD cat = category based on MDD evaluation according to Brock et al. (2015).

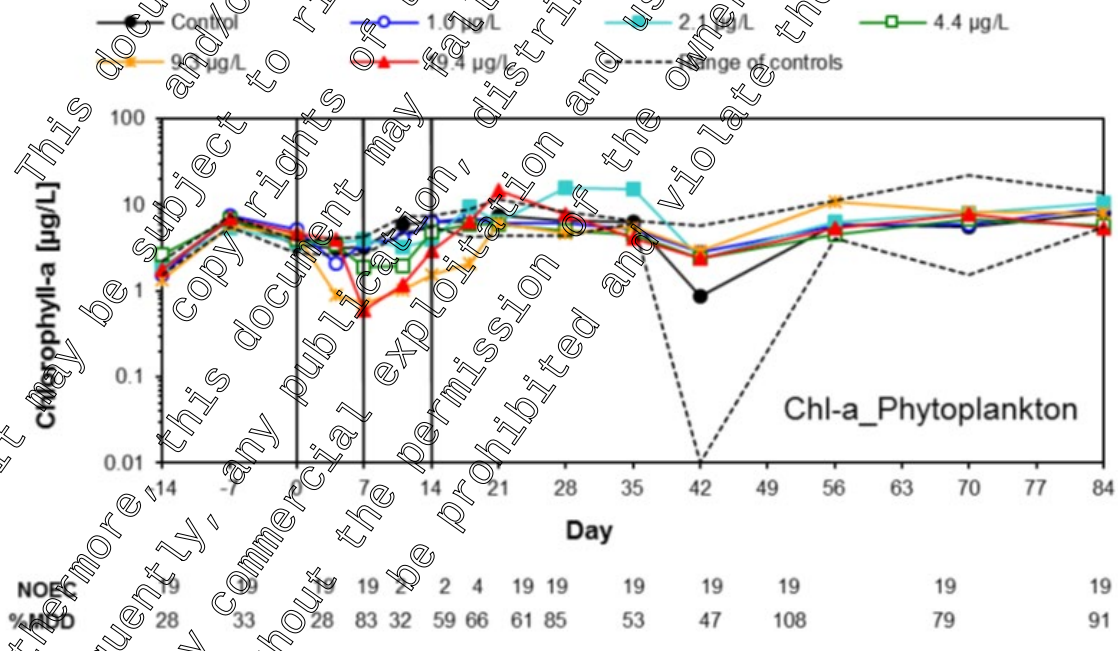
Significantly reduced chlorophyll a concentrations in comparison to the controls were detected on three consecutive samplings, from day 7 to day 14. Therefore, effects at a test concentration of 4.4 µg/L or higher were considered effect class 3A.

Table CP 10.2.3/02-14 NOECs [µg/L] and related % MDDs (in brackets) for phytoplankton chlorophyll a

Phytoplankton		Days after application													
MDD cat	Taxa / day	-14	-7	0	4	7	11	14	18	21	28	42	56	70	84
1	Chl-a Phytoplankton	≥19.4 (28)	≥19.4 (33)	≥19.4 (28)	4.4- (83)	2.1- (32)	2.1- (59)	4.4- (66)	9.3 (46)	≥19.4 (85)	≥19.4 (47)	≥19.4 (108)	≥19.4 (79)	≥19.4 (91)	≥19.4 (56)

Signs indicate the direction of a significant effect and colors indicate the different NOECs. Cat = MDB category according Brock et al. (2015).

Figure CP 10.2.3/02-34 Phytoplankton chlorophyll a: Geometric means per treatment level and range of controls



Periphyton

The effects on periphyton were examined by measurements of chlorophyll a concentrations. The MDDs calculated for periphyton fulfil the criteria according to Brock *et al*. (2015). On most of the sampling occasions, the MDDs were low enough for small to medium effects to be detected. The periphyton

chlorophyll a determinations showed in all test concentrations a slight but not significant difference to the control on day 28 and a significant difference to the control only on day 42. On both dates, the response was not concentration-related since the deviation of the 1.0 µg/L mesocosms from control was very similar to the one of the 19.3 µg/L. Thus, a direct effect of the test item seems to be unlikely (class 1).

Table CP 10.2.3/02-15 % MDDs for periphyton chlorophyll a

Periphyton	Summary			
	Min	Max	Mean	MDD Cat
Chl-a Periphyton	45	99	74	1

MDD cat = category based on MDD evaluation according to Brock et al. (2015)

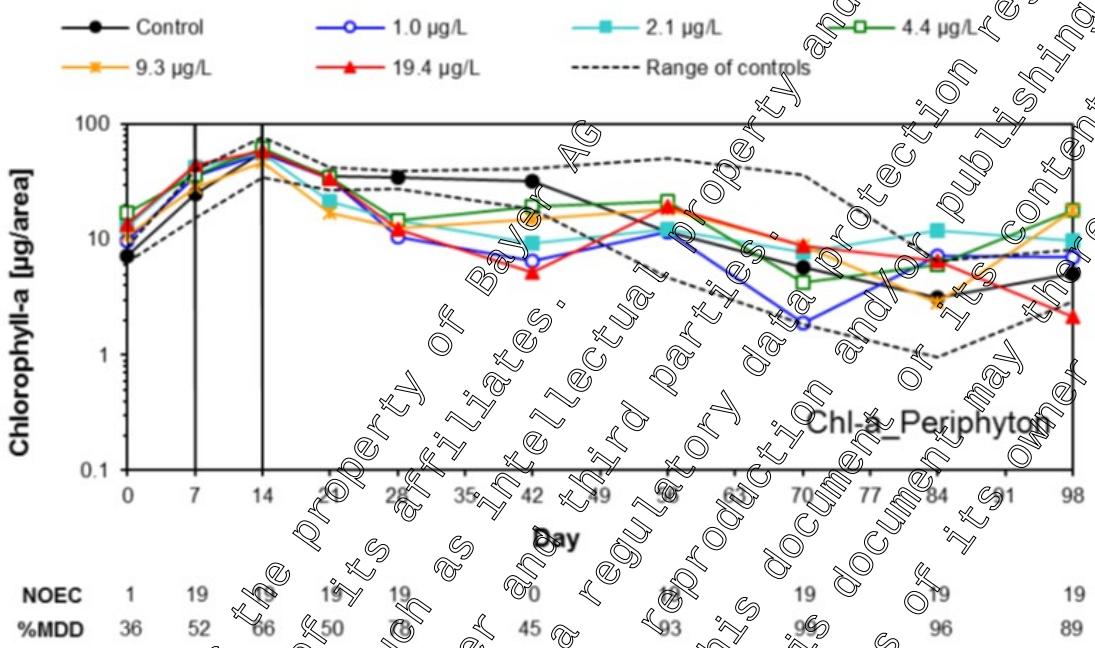
Table CP 10.2.3/02-16 NOECs [µg/L] and related % MDDs (in brackets) for periphyton chlorophyll a

Peryphyton		Days after application								
MD D cat	Taxa / day	0	7	14	21	28	42	56	70	84	98
1	Chl-a Phytoplankton	1+ (36)	≥19.4 (52)	≥19.4 (66)	≥19.4 (50)	≥19.4 (78)	<1-(45)	≥19.4 (93)	≥19.4 (99)	≥19.4 (96)	≥19.4 (89)

Signs indicate the direction of a significant effect and colors indicate the different NOECs.

Cat = MDD category according Brock et al. (2015)

Figure CP 10.2.3/02-35 Periphyton chlorophyll a: Geometric means per treatment level and range of controls



Macrophytes

The calculated MDDs for the total macrophytes coverage were sufficiently low to allow an evaluation of direct effects small to medium effects (MDD 13-60 %) could be determined. No adverse effects on the macrophytes coverage were detected during the outdoor mesocosm study. On day 64 of the study, significantly increased macrophytes coverage was determined at two highest treatments, resulting in a NOEC of 4.4 µg/L.

Table CP 10.2.3/02-17 % MDDs for the taxa in the macrophytes coverage

Macrophytes coverage	Summary			
	Min	Max	Mean	MDD Cat
Coverage %	13	60	27	1

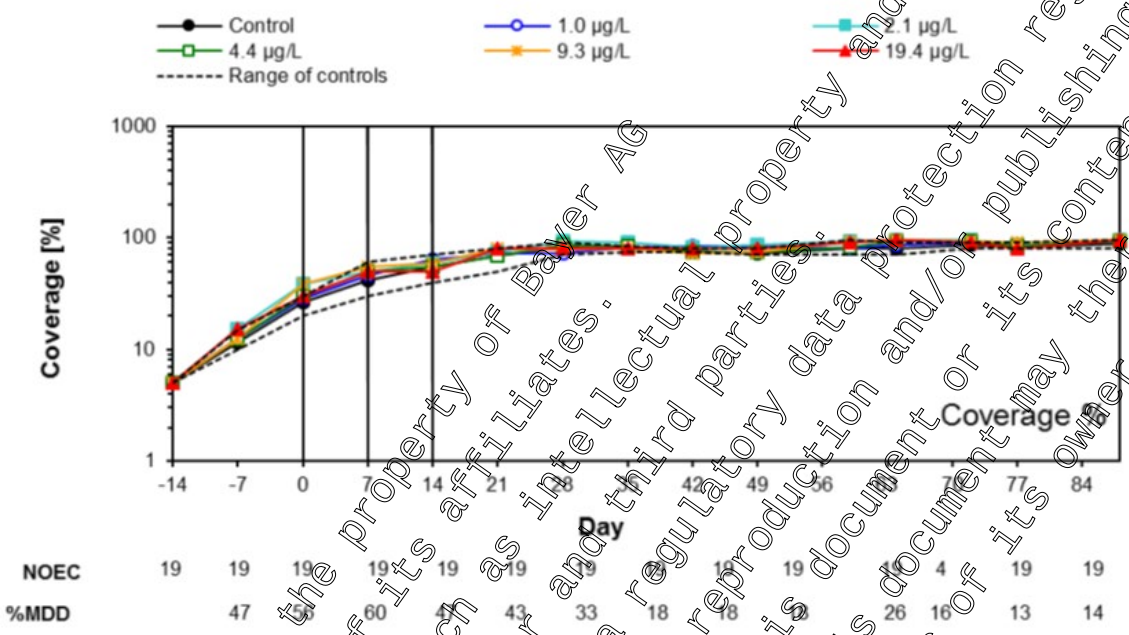
MDD cat = category based on MDD evaluation according to Brock et al. (2015).

Table CP 10.2.3/02-18 NOECs [µg/L] and related % MDDs (in brackets) for macrophytes coverage

Macrophytes coverage		Days after application														
MDD cat	Taxa / day	-14	-7	0	7	14	21	28	35	42	49	59	64	72	77	88
1	Chl-a Phytoplankton	≥19.4 (0)	≥19.4 (47)	≥19.4 (56)	≥19.4 (0)	≥19.4 (47)	≥19.4 (43)	≥19.4 (33)	≥19.4 (18)	≥19.4 (18)	≥19.4 (18)	≥19.4 (26)	4.4+ (16)	≥19.4 (13)	≥19.4 (14)	≥19.4 (13)

Signs indicate the direction of a significant effect and colors indicate the different NOECs. Cat = MDD category according Brock et al. (2015).

Figure CP 10.2.3/02-36 Macrophytes sum of coverage: Geometric means per treatment level and range of controls



III. Conclusions

An outdoor mesocosm study to investigate the effects of three applications of spiroxamine EC 500 was re-analysed with respect to MDDs and effect classification according to the most recent guidance (EFSA 2013).

The MDDs of eleven invertebrate and seven algae taxa (plus combined data on higher taxonomic levels) fulfil the criterion proposed by Brock et al. (2015). If a more strict criterion is applied, *e.g.* that the MDD should be at least once < 70% within the first four weeks after the first application, 13 taxa representing populations of macroinvertebrates, zooplankton and phytoplankton allow a statistical evaluation of direct effects: Tubificidae, Chironomini, *Chaoborus* spec *Simocephalus vetulus*, *Chydorus sphaericus*, *Eucercus lamellatus*, cyclopoid copepods (and nauplia larvae), *Polyarthra spec.*. *Chlamydomonas spec*., coccoid Chlorophyceae, *Chroomonas spec*., *Cryptomonas spec*. (20-30 µm), and Pennales (30-40 µm). Thus, despite the fact that only 12 test systems were used in the study, a reliable statistical analysis of direct effects was possible for at least eight taxa considered to represent potentially sensitive populations as requested by EFSA PPR (2013).

The following effects were found at the different test concentrations:

- At the lowest test concentration of 1.0 µg/L, no treatment effects were found (class 1).
- At 2.1 µg/L, a slight direct effect on total phytoplankton abundance and a pronounced short-term promoting effect on the rotifer Keratella quadrata were detected (class 2 for the direct effect on the phytoplankton and 3A if the potential temporary promotion of a rotifer species is considered an adverse effect). Other taxa showed no effects.
- At 4.4 µg/L, class 3A effects for total rotifers, total phytoplankton, chlorophyll a and Cryptomonas spec. were observed while some other taxa were slightly affected. Thus, the total effect class for 4.4 µg/L is considered 3A.
- At 9.3 µg/L, more taxa were affected, effects were more pronounced or prolonged and for two algae species, significantly higher abundances than in the controls were found at the end of the study. However, since in general both species were rare and no algae bloom was found at the

end of the study, this was not considered to be ecologically relevant. Thus effect class 3A was chosen as the overall effect class for 9.3 µg/L.

- At 19.4 µg/L the effect classification was similar to the one for 9.3 µg/L. For leeches, higher abundances at the end of the study could not be excluded, which was considered as class 2/4A for the highest test concentration.

According to EFSA (2013) the ETO-RAC can be derived from the overall class 1 concentration of 1 µg/L (nominal for three applications) and would be 0.5 µg/L using the recommended assessment factor of 2 in EFSA (2013).

However, if the potential temporary promotion of the rotifer *Keratella* and the slight effect (class 2) on total phytoplankton is considered acceptable, the 2.1 µg/L concentration could be used to derive the ETO-RAC considering that the observed effects here are of low ecological relevance.

Since rotifers and algae seem to be the most sensitive taxa while taxa with a lower recovery potential were found to be less sensitive (Cladocera, Copepoda, Chironomidae, Chaoboridae, Hirudinea, Oligochaeta) the study can also be used to derive an ERO-RAC. At 9.3 µg/L, no, slight or only effects with recovery within 8 weeks were found. Thus, the ERO-RAC can be derived using this concentration (9.3 µg/L) and would be 3.1 µg/L applying an assessment factor of 3.

No clear long-term effects were found at the highest test concentration of at 19.4 µg/L, only for a potential promotion of leeches the effect duration could not be assessed. Thus, the ERO-RAC would be 4.85 µg/L applying an assessment factor of 4 to consider the higher uncertainty due to data on leeches.

Assessment and conclusion by applicant:

The mesocosm study and the re-assessment study has been assessed using the checklist presented in the Aquatic Guidance Document (adapted from De Jong *et al.*, 2008) in order to confirm the reliability of the data. The outcome of the assessment is presented below.

Table CP 10.2.3/02-19 Reliability assessment of the mesocosm study according to EFSA (2013)

Items	Notes	Reliability index 1–3
Methodology and test description		
1. Substance	**Properly characterised and reported?**	
1.1 Concentration	Identity and amount of a.s. per litre test water?	**1** Fully reported (p 19 and 28 of study report0)
1.2 Formulation and purity	Substances in the formulation influencing the working action of the a.s. should be reported	**1** The rep. formulation Spiroxamine EC 500 was tested
1.3 Vehicle	In case a vehicle (other than in the formulation) is used, identity and concentration?	n/a
1.4 Chemical analyses	Method, LOQ, LOD, recovery	**1** Fully reported (p248-262) Refer also to analytical methods section of dossier)
1.5 Properties	Relevant for potential fate and effects in test system	**1**
2. Test site, duration	**Properly characterised and reported?**	
2.1 Location	Necessary to make a link between the effects and local environmental conditions, representativeness	**1** Fully reported (p 21)

2.2 Test date/duration	Application dates and experimental period?	**1** Fully reported (p 25)
2.4 General climatic conditions	Necessary to make a link between the effects and local climatic conditions	**1** Fully reported (p 41)
3. Application	**Properly characterised and reported?**	
3.1 Mode of application	Exposure route; spraying or homogenising the a.s. into the test medium?	**1** Fully reported (p 27)
3.2 Dosage and exposure	Actual concentrations during the test? Chemical analysis of dosing solution?	**1** Fully reported. Chemical analysis of water, sediment and macrophytes
3.3 Application scheme	Necessary to make a link between the test and the intended use of the PPP	**1** Fully reported (p 27)
3.4 Conditions during application	Weather conditions during application, wind speed and temperature?	**1** Fully reported (p 41)
4. Test design	**Properly designed and reported?**	
4.1 Type and size	e.g. outdoor microcosm, outdoor pond or mesocosm; dimensions	**1** Fully reported (p 21 - 22)
4.2 Pre-treatment	Proper equilibration?	**1** 5 months of acclimation prior to dosing
4.3 Treatment period	Number and spacing of treatments?	**1** 3 applications with a 7-day interval
4.3 Post-treatment	Period long enough to allow expression of effects and recovery?	**1** 14 week (84-day) duration sufficient to assess effects and potential recovery
4.4 Untreated control	Sufficient number; solvent applied?	**1** 3 control reps; no solvent required
4.5 Replications	Sufficient replications for proper statistical analysis?	**1** 3 control reps; 2 reps for 1.0, 2.1, 4.4 and 9.3 µg/L; a single rep for 19.4 µg/L
4.6 Statistics	Univariate and multivariate techniques applied?	**1** Fully reported. Also refer to mesocosm re-assessment report)
4.8 Dose–response	Number of test concentrations for finding a dose relationship (excluding controls)	**1** 5 test concentrations used in the study
4.9 Quality assurance	Study conducted under GLP?	**1** GLP study
5. Biological system	**Representative and properly reported?**	
5.1 Populations	Enough sensitive/vulnerable species of the relevant taxonomic group?	**1** MDD criteria fulfilled (at least 8 sensitive taxa). Refer to re-assessment report

5.2 Community	The community/ecosystem representative and complete?	**1** Aquatic ecosystem considered sufficiently represented
6. Sampling	**Is sampling adequate for risk assessment?**	
6.1 General features	Relevance selected measurement endpoints	**1** Fully reported (p 29 – 32)
6.2 Actual concentration	Actual concentrations measured in medium and other compartments or biota?	**1** Fully reported; concentrations measured in overlying water, sediment and in macrophytes
6.3 Biological sampling	Appropriate methods and frequency?	**1** Suitable sampling techniques used for zooplankton, macrozoobenthos, chlorophyll a, algae and macrophytes (p 29 – 32)
Results		
7. Endpoints	**Properly reported?**	
7.1 Type	Reported endpoints relevant for objective of study?	**1** Fully reported. Also refer to re-assessment report
7.2 Value	Are measured data consistently presented?	**1**
7.3 Verification of endpoint	Test results are verifiable and source data reported	**1** Fully reported (refer to re-assessment report)
8. Elaboration of results	**Are conclusions based on measured data? Methodology correct?**	
8.1 Statistical comparison	Data meet requirements for method used?	**1** (refer to re-assessment report)
8.2 Dose–effect relationship	Minimal detectable difference; consistence of response	**1** MDD analysis performed (refer to re-assessment report)
8.3 Population-level responses	Sufficiently reported?	**1** Sufficiently reported
8.4 Community-level responses	Sufficiently reported?	**1** Sufficiently reported
9. Control		
9.1 Untreated control	Unexpected effects or disappearance of species?	**1** No unexpected effects
9.2 Solvent control	Possible effects caused by solvent?	**1** No solvent used
10. Classification of effects	**Properly derivable?**	**1** Refer to re-assessment report where effects are classified in accordance with the Aquatic Guidance Document

11. Biological meaning of statistically significant differences	Sufficiently explained?	1 Fully reported

Relevant page numbers from the mesocosm report (FYF 379) have been added for information purposes.

1 Reliable - All data are reported, the methodology and the description are in accordance with internationally accepted test guidelines and/or the instructions, all other requirements fulfilled

2 Less Reliable - Not all data reported, the methodology and/or the description are slightly deviating from internationally accepted test guidelines or the instructions, without motivation, or not all other requirements fulfilled

3 Not reliable - Essential data missing, the methodology and/or the description are not in accordance with internationally accepted test guidelines and/or the instructions without motivation, or not reported or important other requirements are not fulfilled

Based on the reliability assessment above, it is considered that the mesocosm study was conducted to recognised test methodology and was sufficiently reported. Taking the results of this study as well as the results of the re-assessment study into account, it is considered that the data are robust and reliable and of sufficient quality to be able to derive an endpoint for use in the aquatic risk assessment.

For phytoplankton, macroinvertebrates and zooplankton, 19 taxa plus pooled data on higher taxonomic levels fulfilled the MDD criterion proposed by Brock *et al.* (2015). Furthermore, the chlorophyll a measurements of phytoplankton and periphyton as well as the macrophyte coverage could be evaluated. If a more strict criterion is applied, *e.g.* that the MDD should be at least once < 70% within the first four weeks after the first application, 13 taxa representing populations of macroinvertebrates, zooplankton and phytoplankton allow a statistical evaluation of direct effects: Tubificidae, Chironomini, *Chaoborus spec Simocephalus vetulus, Chydorus sphaericus, Eucercus lamellatus*, cyclopoid copepods (and nauplia larvae), *Polyarthra spec., Chlamydomonas spec.*, coccoid Chlorophyceae, *Chroomonas spec., Cryptomonas spec.* (20-30 µm), and Pennales (30-40 µm). Thus, the requirement of the Aquatic Guidance Document (EFSA PPR 2013) that the MDDs should be sufficiently low to allow the analysis of direct effect for at least 8 potential sensitive populations is met by the study.

The study is considered to be acceptable and sufficiently robust to derive an ETO-RAC of 0.5 µg a.s./L (Class 1 effects at 1.0 µg a.s./L and an assessment factor of 2). The data are also considered sufficient to derive an ERO-RAC of 3.1 µg a.s./L (Class 3A effects at 9.3 µg a.s./L and an assessment factor of 3).

CP 10.3 Effects on arthropods

CP 10.3.1 Effects on bees

The available data for spiroxamine technical with bees are presented in Table 10.3.1-1 below. The available data for prothioconazole are presented in Table 10.3.1-2 and the available data for Prothioconazole + Spiroxamine EC 460 are presented in Table 10.3.1-3.

Table CP 10.3.1-1 Summary of bee toxicity studies with spiroxamine

Organism	Test item	Test type	Endpoints		Reference
Adult honey bee (*Apis mellifera*)	Spiroxamine	Acute oral	48 h LD_{50} >100 µg a.s./bee	EU	M-008208-01-1
Adult bumble bee (*Bombus terrestris*)	Spiroxamine	Acute oral	48 h LD_{50} >50.9 µg a.s./bumblebee	NEW	M-688128-01-1
Adult honey bee (*Apis mellifera*)	Spiroxamine	Acute contact	48 h LD_{50} 4.2 µg a.s./bee	EU	M-008208-01-1

Organism	Test item	Test type	Endpoints		Reference
Adult bumble bee (*Bombus terrestris*)	Spiroxamine	Acute contact	48 h LD_{50} >100 µg a.s./bumblebee	NEW	M-510841-01-1
Honey bee larva (*Apis mellifera*)	Spiroxamine	Chronic larva (22 day repeated exposure)	LD_{50} >33 µg a.s./larva NOED 33 µg a.s./larva	NEW	M-623462-01-1

EU: previously evaluated as part of the original EU review and listed in EFSA conclusion and DAR
NEW: new study or data generated since the previous EU review or previously not submitted
Values in **bold** have been used in the risk assessment

Table CP 10.3.1-2 Summary of bee toxicity studies with prothioconazole

Organism	Test item	Test type	Endpoints		Reference
Adult honey bee (*Apis mellifera*)	Prothioconazole	Acute oral	LD_{50} >71 µg a.s./bee	EU	EFSA Conclusion[1]
Adult honey bee (*Apis mellifera*)	Prothioconazole	Acute contact	LD_{50} >200 µg a.s./bee	EU	

EU: previously evaluated as part of the original EU review and listed in EFSA conclusion and DAR
[1] EFSA Scientific Report (2007) 106, 1-98. Conclusion on the peer review of prothioconazole

Table CP 10.3.1-3 Summary of bee toxicity studies with Prothioconazole + Spiroxamine EC 460

Organism	Test item	Test type	Endpoints		Reference
Adult honey bee (*Apis mellifera*)	Prothioconazole + Spiroxamine EC 460	Acute oral	**48 h LD_{50} 346 µg product/bee**	EU	M-074353-01-1
Adult bumble bee (*Bombus terrestris*)	Prothioconazole + Spiroxamine EC 460	Acute oral	48 h LD_{50} >200.8 µg product/ bumblebee	NEW	M-704649-01-1
Adult honey bee (*Apis mellifera*)	Prothioconazole + Spiroxamine EC 460	Acute contact	**48 h LD_{50} 420 µg product/bee**	EU	M-074353-01-1
Adult bumble bee (*Bombus terrestris*)	Prothioconazole + Spiroxamine EC 460	Acute contact	48 h LD_{50} >400 µg product/ bumblebee	NEW	M-704649-01-1
Adult honey bee (*Apis mellifera*)	Prothioconazole + Spiroxamine EC 460	Chronic oral	10-day LC_{50} 2003.9 mg product/kg feeding solution 10-day LDD_{50} 26.8 µg product/bee/day **NOEDD 15.0 µg product/bee/day**	NEW	M-690829-01-1

Organism	Test item	Test type	Endpoints		Reference
Adult honey bee (*Apis mellifera*)	Prothioconazole + Spiroxamine EC 460	Chronic oral	10-day LC_{50} >672 mg product/kg feeding solution 10-day LDD_{50} >13.9 µg product/bee/day NOEDD 9.52 µg product/bee/day	NEW	M-755308-01-1
Honey bee larva (*Apis mellifera*)	Prothioconazole + Spiroxamine EC 460	Chronic larva (22 day emergence)	ED_{50} 151.6 µg product/larva **NOED 48 µg product/larva**	NEW	M-756625-01-1

EU: previously evaluated as part of the original EU review and listed in EFSA conclusion and DAR
NEW: new study or data generated since the previous EU review or previously not submitted
Values in **bold** have been used in the risk assessment

Exposure

The highest single application rate of Prothioconazole + Spiroxamine EC 460 to cereals is 1.25 L product/ha. This rate has been considered below in the risk assessment for bees.

Selection of endpoints

The EFSA Conclusion for prothioconazole (EFSA Scientific Report (2007) 106, 1-98) provides acute honeybee endpoints for prothioconazole technical of >71 and 200 µg a.s./bee for oral and contact toxicity, respectively (refer to Table 10.3.1-2). Formulation (250 EC) specific values are also provided of >48.7 and >200 µg a.s./bee for oral and contact toxicity, respectively, which are not presented above as these values are not considered relevant to the risk assessment of Prothioconazole + Spiroxamine EC 460. For the risk assessment below it is not considered appropriate to use any of the endpoints determined for prothioconazole because the endpoints determined for Prothioconazole + Spiroxamine EC 460 are considered to be the most relevant.

For the acute risk assessment the oral and contact LD_{50} values have been determined to be 346 and 420 µg product/bee, respectively in the study conducted with Prothioconazole + Spiroxamine EC 460.

For the chronic adult oral endpoint, two valid studies with Prothioconazole + Spiroxamine EC 460 are available. In the first study (M-755308-01-1) the LDD_{50} was determined to be >13.9 µg product/bee/day as there was only 30% mortality recorded at the highest tested dose of 13.9 µg product/bee/day. The NOEDD in this study was determined to be 9.52 µg product/bee/day. In a second study (M-690829-01-1) a bound LDD_{50} value of 26.8 µg product/bee/day was established with a NOEDD of 15.0 µg product/bee/day. The chronic risk assessment has therefore taken the lowest NOEDD of 9.52 µg product/bee/day.

For larval toxicity a 22-day repeated exposure study with Prothioconazole + Spiroxamine EC 460 is available which gave a NOED of 48 µg product/larva.

Isomers

The risk assessments for bees involves potential chronic exposure of these organisms to residues in plants therefore it may be necessary to apply an uncertainty factor (UF) to the chronic risk assessments. The acute risk assessment need not have an UF applied as exposure in this scenario is immediate but chronic risk assessment considers exposure over a prolonged period therefore potential changes in isomeric ratio needs to be considered. For the bee risk assessments the same approach taken in the residues section for the consumer risk assessment, with respect to isomers, has been followed. Based on the current residues data set for spiroxamine, there are no indications of a significant change in isomer

ratios therefore no additional factor need be applied to the risk assessments below (*i.e.* an UF of 1.0 has been used).

Risk assessment for bees

The EFSA[9] guidance on bee risk assessment has not been noted at the EU level and is currently under revision. The notifier has therefore presented an acute risk assessment in accordance with the current SANCO[10] terrestrial guidance document. However, in order to consider the chronic risks to bees, an illustrative assessment of chronic risk has been presented using the existing EU community guidance provided by EPPO (2010)[11].

CP 10.3.1-4
Calculation of HQ_{oral} for honey bees exposed to Prothioconazole + Spiroxamine EC 460

Test substance	Crop Group	Species	App. rate (g product/ha)	LD_{50} oral (µg product/bee)	HQ_{oral}	Trigger
Prothioconazole + Spiroxamine EC 460	Cereals BBCH 30-69	Honeybee	1230*	346	3.55	50

HQ (Hazard Quotient) for adult oral exposure
Application rate converted from L product/ha to g product/ha using a formulation density of 0.984 g/cm^3 from study M-074353-01-1

CP 10.3.1-5
Calculation of $HQ_{contact}$ for honey bees exposed to Prothioconazole + Spiroxamine EC 460

Test substance	Crop Group	Species	App. rate (g product/ha)	LD_{50} contact (µg product/bee)	$HQ_{contact}$	Trigger
Prothioconazole + Spiroxamine EC 460	Cereals BBCH 30-69	Honeybee	1230*	420	2.93	50

HQ (Hazard Quotient) for adult contact exposure
Application rate converted from L product/ha to g product/ha using a formulation density of 0.984 g/cm^3 from study M-074353-01-1

The HQ values for both oral and contact exposure to honey bees are below the trigger value of 50 thereby demonstrating acceptable risks to bees following the proposed use of Prothioconazole + Spiroxamine EC 460 to cereals at 1.25 L product/ha.

Chronic toxicity to honey bees

A chronic risk assessment has been presented below in accordance with the EPPO scheme. Although the EPPO guidance gives procedures based on systemic product applied by soil or seed treatments, the methodology is also suitable for the chronic risk by spray application.

[9] European Food Safety Authority, 2013 (*updated 04 July 2014*). EFSA Guidance Document on the risk assessment of plant protection products on bees (*Apis mellifera*, *Bombus* spp. and solitary bees). EFSA Journal 2013;11(7):3295, 268 pp., doi:10.2903/j.efsa.2013.3295
[10] SANCO/10329/2002 rev 2 final (17 October 2002). Guidance Document on Terrestrial Ecotoxicology Under council Directive 91/414/EEC
[11] EPPO 2010: EPPO Standard PP 3/10 (3) Environmental risk assessment scheme for plant protection products. Chapter 10: honeybees

The chronic risk assessment below considers exposure *via* pollen only. Cereals, such as wheat, are wind pollinated and do not rely on bees as a source of pollination. As a result, the flowers tend not to produce nectar in order to attract bees. Thus, nectar is not considered to be a viable route of exposure to bees from cereals.

The chronic risk assessment for adult honey bees and honey bee larvae is based on the generic worst-case residue value of 1 mg a.s./kg plant matrix in pollen, as specified in the revised EPPO scheme (2010) and determines the ratio between the NOEDD (oral) and the exposure by means of a TER calculation. For adult honey bees, the exposure was assessed through the amount of residues that may be ingested by a bee in one day. The ratio between the NOEDD (µg a.s./bee/day) and the exposure (in µg a.s./bee/day) was calculated using the following formula:

$$TER_{chronic,adult} = \frac{NOEDD_{oral} (\mu g\ a.s./bee/day)}{Residues\ ingested\ by\ a\ bee\ in\ one\ day\ (\mu g\ a.s./bee/day)}$$

As the available endpoint for larvae is expressed over the total developmental period, the exposure for larvae was assessed through the amount of residues that may be ingested by the larvae over that period. For larvae, the ratio between the NOED (in µg a.s./larva) and the exposure (in µg a.s./larva) was calculated using the following formula:

$$TER_{chronic,larvae} = \frac{NOED_{oral} (\mu g\ a.s./larva)}{Residues\ ingested\ by\ a\ larva\ (\mu g\ a.s./larva)}$$

Data for consumption of nectar and pollen by adult honey bees and honey bee larvae are given in the EFSA Opinion on bees (2012)[12]. According to the EFSA Opinion the maximum amount of pollen an adult bee consumes per day is 300 mg/bee/day. For honey bee larvae the maximum amount of pollen consumed by a larva is stated as 2 mg/5 days.

To calculate the residue intake of spiroxamine by adult honey bees and honey bee larvae, the consumed amounts of pollen are multiplied with the generic residue value in nectar and pollen of 1.0 mg a.s./kg (equivalent to 0.001 µg a.s./mg). However, as the risk assessment is conducted for a mixture formulation comprising two actives, a residue value of 2.0 mg a.s./kg (*i.e.* 1.0 + 1.0) has been assumed. Thus, adult honey bees that consume 300 mg/bee/day of pollen will therefore be exposed to 0.6 µg a.s./bee/day (300 mg/bee/day x 0.002 µg a.s./mg). Larvae will be exposed to 0.004 µg a.s./larva through the consumption of pollen.

TER values have been calculated in the table below and are compared to the TER trigger value of 1, as stated in the EPPO scheme. In order to determine the TER values, the toxicity endpoints have been converted from µg product to µg total a.s.. Thus, the NOEDD from the chronic adult oral study of 9.52 µg product/bee/day is equivalent to 4.41 µg total a.s./bee/day and the NOED of 48 µg product/larva is equivalent to 22.2 µg total a.s./larva. The calculations are based on a spiroxamine content of 30.1% w/w and a prothioconazole content of 16.2% w/w giving a total a.s. content of 46.3% w/w.

CP 10.3.1-6

Chronic risk assessment for honeybee adults and larvae from exposure to Prothioconazole + Spiroxamine EC 460 *via* pollen

Stage of development	Route of exposure	NOED	Residue intake	TER	Trigger value

[12] EFSA Panel on Plant Protection Products and their Residues (PPR) (2012). Scientific Opinion on the science behind the development of a risk assessment of Plant Protection Products on bees (Apis mellifera, Bombus spp. and solitary bees). EFSA Journal 2012; 10(5) 2668

Adult	Oral	4.41 µg total a.s./bee/day	0.6 µg total a.s./bee/day	7.35	1
Larvae		22.2 µg total a.s./larva	0.004 µg total a.s./larva	5550	

The TER values are both greater than the trigger value of 1 therefore the chronic risks to honey bee adults and larvae, from consumption of potentially contaminated pollen, are considered to be acceptable. Thus, the chronic risks to bees following the proposed uses of Prothioconazole + Spiroxamine EC 460 on cereals are considered to be acceptable.

Bumble bee data

Acute oral and contact toxicity data using Prothioconazole + Spiroxamine EC 460 are available for bumble bees. The acute oral LD_{50} has been established to be > 200.8 µg product/bumble bee and the acute contact LD_{50} has been established to be >400 µg product/bumble bee. The data demonstrates that bumble bees can be considered to be no more acutely sensitive than honey bees to the effects of Prothioconazole + Spiroxamine EC 460.

Higher-tier risk assessment

A higher-tier risk assessment is not considered to be necessary as acceptable acute and chronic risks have been demonstrated in the risk assessments above. Residues decline data in nectar and pollen are available for spiroxamine (M-763122-01-1). In the study, Spiroxamine EC 500 was applied twice to *Phacelia tanacetifolia* (at pre-flowering and flowering growth stages) in semi-field tunnel conditions at a rate of 300 g a.s./ha with a 10-day interval. Two trials were conducted in Germany and three trials in Spain. Sampling occurred shortly after the second application, 8 hours, 1, 2, 3, 5 and 7 days after the second application. The study has been summarized in Section CP 10.3.1.5 below. The results confirm that residues of spiroxamine dissipated relatively quickly following application. These results are considered to be suitable for use in a refined risk assessment, where required.

Biodiversity

No relevant scientifically peer-reviewed open literature could be found on spiroxamine or its major metabolites, from an ecotoxicological perspective, on bees. Therefore, it is considered that the potential impact of the active substance on biodiversity and the ecosystem, including potential indirect effects *via* alteration of the food web, are covered by the risk assessment for bees in this section.

The risk assessment for bees does not indicate a need for higher tier assessment nor mitigation measures. Therefore, the applicant concludes that the use of the representative lead formulation Prothioconazole + Spiroxamine EC 460 has low potential to cause unacceptable effects on biodiversity and the ecosystem *via* trophic interactions. To the best of our knowledge and with the presented safety profile of the active substance spiroxamine and the representative lead formulation, the applicant does not foresee any effects on biodiversity and the ecosystem.

CP 10.3.1.1 Acute toxicity to bees

CP 10.3.1.1.1 Acute oral toxicity to bees

Data Point:	KCP 10.3.1.1.1/01
Report Author:	[redacted]
Report Year:	2001
Report Title:	Acute toxicity of JAU 6476 & spiroxamine EC 460 to the honeybee Apis mellifera L. under laboratory conditions
Report No:	01 10 48 033
Document No:	M-074353-01-1
Guideline(s) followed in study:	OECD no. 213 (1998) OECD no. 214 (1998)
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and accepted RAR (2010)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

Honeybees, *Apis mellifera carnica* L., were exposed to JAU 6476 + Spiroxamine EC 460 in oral and contact toxicity tests over 48-hours.

The test item was applied at concentrations of 6.31, 12.53, 25.06, 50.11, 100.23 and 200.46 µg total a.s./bee in the oral toxicity test and at concentrations of 12.53, 25.06, 50.11, 100.23 and 200.46 µg total a.s./bee in the contact toxicity test. These rates were equivalent to 13.6, 27.0, 54.0, 108, 216 and 432 µg product/bee for the oral test and 27.0, 54.0, 108, 216 and 432 µg product/bee for the contact test. Dimethoate EC 400 was used as a toxic standard.

The calculated 48-hour LD_{50} values were 161 and 195 µg total a.s./bee in the oral and contact toxicity tests, respectively (equivalent to 346 and 420 µg product/bee in the oral and contact toxicity tests, respectively).

I. Materials and Methods

Materials

Test Material	JAU 6476 + Spiroxamine EC 460
Lot/Batch #:	06920/0045(0019)
Purity:	JAU 6476: 160.4 g/L Spiroxamine: 296.2 g/L
Description:	Clear, dark yellow liquid
Stability of test compound:	Not reported
Reanalysis/Expiry date:	2 November 2001
Density:	0.984 g/cm³

Treatments

Test rates:	Oral: 6.31, 12.53, 25.06, 50.11, 100.23 and 200.46 µg total a.s./bee (equivalent to 13.6, 27.0, 54.0, 108, 216 and 432 µg product/bee) Contact: 12.53, 25.06, 50.11, 100.23 and 200.46 µg total a.s./bee (equivalent to 27.0, 54.0, 108, 216 and 432 µg product/bee)

Solvent/vehicle:	Oral test: sucrose solution Contact test: acetone
Analysis of test concentrations:	No
Test organisms	
Species:	Honeybee, *Apis mellifera carnica* L.
Source:	Purchased from beekeeper [redacted]
Acclimatisation period:	1-2 hours
Feeding:	Continuously during the test with 50% (w/v) aqueous sucrose solution
Treatment for disease:	None reported
Test design	
Test vessel:	Disposable cage of cardboard with holes in the bottom for ventilation and a glass plate in front for observation of the bees (dimensions inside: 80 x 45 x 65 mm)
Replication:	3 per test concentration
No. animals/vessel:	10
Duration of test:	48 hours
Environmental test conditions	
Temperature:	25°C ± 2°C
Relative humidity:	64 - 75%
Photoperiod:	Constant darkness

Study Design

Worker bees of the honeybee, *Apis mellifera* L., were exposed to different doses of JAU 6476 + Spiroxamine EC 460 and the reference item. The treated bees were kept under controlled climatic conditions and assessed for toxic effects for up to 48 hours.

The test item was applied at concentrations of 6.31, 12.53, 25.06, 50.11, 100.23 and 200.46 µg total a.s./bee in the oral toxicity test and at concentrations of 12.53, 25.06, 50.11, 100.23 and 200.46 µg total a.s./bee for the contact toxicity test.

Dimethoate EC 400 was used as a toxic standard. For the reference oral test rates of 0.074, 0.089, 0.104, 0.126 and 0.149 µg a.s./bee were tested and for the reference contact test rates of 0.012, 0.023, 0.046, 0.093 and 0.186 µg a.s./bee were tested.

The honeybees were kept in disposable cages made of cardboard with holes in the bottom for ventilation and a glass plate in front for observation of the bees (dimensions inside: 80 x 45 x 65 mm). Ten bees were housed in each vessel and three replicates of each test concentration were conducted.

For the oral toxicity test, the bees were fed with 50% aqueous sucrose solution that contained either the test item or the reference item. The control treatments were fed with 50% aqueous sucrose solution. The amount of solution ingested by the bees was approximately 20 µL/bee.

For the contact toxicity test, the test item and reference items were dissolved in acetone. Bees were anaesthetised with CO_2 and were treated individually by topical application of 1 µL of the test solution to the dorsal thorax of each bee. In the control treatment groups, 1 µL pure water and 1 µL of untreated acetone, respectively, were applied in the same manner.

The bees were kept at 25 ± 2°C and in continuous darkness. Conditions were measured continuously with a data logger.

The number of dead and affected bees were counted at 4, 24 and 48 hours. At these times, any behavioural abnormalities of the bees were also recorded.

II. Results and Discussion

Validity criteria according to the OECD 213 (1998) and OECD 214 (1998) guidelines, to which the study was conducted, were met:

- The average mortality in the controls to be < 10% (actual: 0% in both oral and contact tests)
- The 24-hour LD_{50} of the toxic standard should be within the range of 0.10 - 0.35 µg a.s./bee (oral) and 0.10 - 0.30 µg a.s./bee (contact). (Actual: oral: 0.146 µg a.s./bee and contact: 0.136 µg a.s./bee)

Statistically significant effects on survival were observed at doses of 100.23 and 200.46 µg total a.s./bee in the oral toxicity test (23.3% and 63.3% mortality, respectively) after 48 hours exposure.

Statistically significant effects on survival were observed only at a dose of 200.46 µg total a.s./bee in the contact toxicity test (63.3% mortality) after 48 hours exposure.

Sub-lethal effects of apathy and immobility were observed prior to death in affected bees.

Table CP 10.3.1.1.1/01-1 Mortality of honeybees exposed to JAU 6476 + Spiroxamine EC 460 over 48 hours

Dose (µg total a.s./bee)	% mortality (oral)	% mortality (contact)
Control	0	0
6.31	0	-
12.53	0	6.7
25.06	0	0
50.11	3.3	10.0
100.23	23.3*	20.0
200.46	63.3*	63.3*

- Not tested
* statistically significantly increased according to Dunnett's test ($p \leq 0.05$, one-sided smaller)

Table CP 10.3.1.1.1/01-2 LD_{50} values determined in the acute oral and contact toxicity tests with JAU 6476 + Spiroxamine EC 460

Time	LD_{50}			
	Oral toxicity test		Contact toxicity test	
	µg total a.s./bee	µg product/bee	µg total a.s./bee	µg product/bee
24-hour	171 (134 - 217)	368 (290 – 469)	178 (129 – 244)	383 (279 – 526)
48-hour	161 (128 – 201)	346 (277 – 433)	195 (147 – 324)	420 (252 – 699)

Values in parentheses are 95% confidence limits

Table CP 10.3.1.1.1/01-3 LD_{50} values determined in the acute oral and contact toxicity tests with the reference item Dimethoate EC 400

Time	LD_{50}			
	Oral toxicity test		Contact toxicity test	
	µg a.s./bee	µg product/bee	µg a.s./bee	µg product/bee
24-hour	0.146 (0.128 – 0.168)	0.390 (0.345 – 0.452)	0.136 (0.095 – 0.194)	0.366 (0.256 – 0.522)
48-hour	0.141 (0.124 – 0.159)	0.380 (0.334 – 0.428)	0.099 (0.079 – 0.124)	0.267 (0.213 – 0.334)

Values in parentheses are 95% confidence limits

III. Conclusion

Honeybees (*Apis mellifera* L.) were exposed to JAU 6476 + Spiroxamine EC 460 in a 48-hour oral and contact toxicity study.

The calculated 48-hour LD_{50} value in the oral toxicity test was 161 µg total a.s./bee (equivalent to 346 µg product/bee).

The calculated 48-hour LD_{50} value in the contact toxicity test was 195 µg total a.s./bee (equivalent to 420 µg product/bee).

Assessment and conclusion by applicant:

Validity criteria according to the OECD 213 (1998) and OECD 214 (1998) guidelines, to which the study has been conducted, were met:

- The average mortality in the controls to be <10% (actual: 0% in both oral and contact tests)
- The 24-hour LD_{50} of the toxic standard should be within the range of 0.10 - 0.35 µg a.s./bee (oral) and 0.10 - 0.30 µg a.s./bee (contact). (Actual: oral: 0.146 µg a.s./bee and contact: 0.136 µg a.s./bee)

The study is therefore considered acceptable.

The study reported results in terms of total active substance as well as formulation. Thus, in terms of spiroxamine content the calculated 48-hour LD_{50} value in the oral toxicity test was 104 µg a.s./bee and the calculated 48-hour LD_{50} value in the contact toxicity test was 126 µg a.s./bee. In terms of product content the calculated 48-hour LD_{50} value in the oral toxicity test was 346 µg product/bee and the calculated 48-hour LD_{50} value in the contact toxicity test was 420 µg product/bee.

Data Point:	KCP 10.3.1.1.1/02
Report Author:	[redacted]; [redacted]
Report Year:	2020
Report Title:	Prothioconazole + spiroxamine EC 460: Effects (acute contact and oral) on bumblebees (Bombus terrestris L.) in the laboratory
Report No:	143101105
Document No:	M-704649-01-1
Guideline(s) followed in study:	Regulation (EC) No. 1107/2009 Directive 2003-01 (Canada/PMRA) US EPA OCSPP 850.3020, 850.supp. OECD 246 and 247 (2017)
Deviations from current test guideline:	None
Previous evaluation:	No, not previously submitted -
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

Bumblebees (*Bombus terrestris* L.) were exposed to Prothioconazole + Spiroxamine EC 460 in a 48-hour contact toxicity test and a 48-hour oral toxicity test.

Exposure was at dose levels of 400, 200, 100, 50 and 25 µg product/bumblebee in the contact test and at dose levels of 320, 160, 80, 40 and 20 µg product/bumblebee in the oral test. In the oral test actual dose rates of 200.8, 139.4, 78.4, 40.4 and 22.6 µg product/bumblebee were achieved.

In the contact test, 10 µg dimethoate/bumblebee was used as a reference item along with a water control. In the oral test, 4.5 µg dimethoate/bumblebee was used as a reference item along with a water control. This was in accordance with the OECD 246 and 247 (2017) guidelines.

The 48-hour NOED and LD_{50} values for the contact test were ≥400 and >400 µg product/bumblebee, respectively.

The 48-hour NOED and LD_{50} values for the oral test were ≥200.8 and >200.8 µg product/bumblebee, respectively.

I. Materials and Methods

Materials

Test Material Prothioconazole + Spiroxamine EC 460

Lot/Batch #: EM4L025614

Purity: Prothioconazole: 16.2% w/w, 159.0 g/L
Spiroxamine: 30.1% w/w, 295.7 g/L

Description: Yellow-brown liquid

Reanalysis/Expiry date: 08 April 2021

Density: 0.983 g/mL

Treatments

Test rates: Contact: 400, 200, 100, 50 and 25 µg product/bumblebee

	Oral (nominal): 320, 160, 80, 40 and 20 µg product/bumblebee Oral (actual): 200.8, 139.4, 78.4, 40.4 and 22.6 µg product/bumblebee
Solvent/vehicle:	Contact: Triton X-100 (0.1%) in water Oral: 50% w/v sucrose solution
Analysis of test concentrations:	Yes. Analysis of highest and lowest application solutions
Test organisms	
Species:	Adult female worker bumblebees, *Bombus terrestris* L., Insecta, Hymenoptera
Source:	Koppert Deutschland GmbH, Zeppelinstr. D-47638 Straelen
Acclimatisation period:	Contact: 20 hours and 10 minutes Oral: 41 hours and 50 minutes
Feeding:	50% w/v sucrose solution *ad libitum*
Test design	
Test vessel:	Cylindrical, latticed plastic cages (7.3 cm x 2.2 cm x 1.7 cm) with a bottom plate
Replication:	Contact: 30 per each treatment group, 30 for the water control and 30 for the reference item group Oral: 30 per each treatment group, 30 for the water control and 30 for the reference item group
No. animals/vessel:	1 per test vessel
Duration of test:	Contact: 48 hours Oral: 48 hours
Environmental test conditions	
Temperature:	Contact: 24.9 – 25.3°C Oral: 25.2 – 25.4°C
Relative humidity:	Contact: 50.7 – 74.2% Oral: 50.8 – 64.6%
Photoperiod:	Darkness (except during observation)

Study Design

Bumblebees were exposed to Prothioconazole + Spiroxamine EC 460 in acute contact and oral tests over 48 hours each.

The test organisms were adult female worker *Bombus terrestris* L. obtained from Koppert Deutschland GmbH, Straelen. The bumblebees were kept in test units and the contact application was conducted outside of the test unit. Temperature and relative humidity in the contact test were kept within 24.9 to 25.3°C and 50.7 to 74.2%, respectively throughout the test period. Temperature and relative humidity in the oral test were kept within 25.2 to 25.4°C and 50.8 to 64.6%, respectively throughout the test period. The bees were kept in darkness except during observation.

During both tests, the bees were individually housed in cylindrical, latticed plastic cages (7.3 cm x 2.2 cm x 1.7 cm) placed on a bottom plate. Thirty replicates per each treatment group, the water control and the reference item were used in the test.

In both tests, bees were anaesthetised with CO_2 until they were immobilised for weighing (contact test) and for weighing and application (oral test). In the oral test, the bees were fasted for 200 to 233 minutes prior to application.

In the contact test, thirty bees were treated with each concentration of the test item of 400, 200, 100, 50 and 25 µg product/bumblebee, a water control and the reference item for 48 hours. In the oral test, thirty bees were treated with each concentration of the test item of 320, 160, 80, 40 and 20 µg product/bumblebee, a water control and the reference item for 48 hours. In the contact test, 10 µg dimethoate/bumblebee was used as a reference item along with a water control. In the oral test, 4.5 µg dimethoate/bumblebee was used as a reference item along with a water control.

In the contact test, the test item was applied as one 2 µL droplet of Prothioconazole + Spiroxamine EC 460, diluted in tap water with 0.1% v/v Triton X-100 on the dorsal thorax using a pipette. The reference item was applied as one 2 µL droplet of dimethoate, diluted in tap water with 0.1% v/v Triton X-100. For the control, one 2 µL droplet of tap water containing 0.1% v/v Triton X-100 was used.

In the oral test, the test item was applied in 50% w/v sucrose solution. The reference item was applied in 50% w/v sucrose solution and for the water control, pure 50% w/v was used. Approximately 40 µL food solution per bumblebee was provided in syringes which were weighed before and after introduction into the cages in order to determine food consumption.

In both tests, mortality was observed at 4 (± 0.5), 24 (± 2) and 48 (± 2) hours. Behavioural abnormalities (*e.g.* moribund) were observed at 4 (± 0.5), 24 (± 2) and 48 (± 2) hours.

In the oral test bumblebees which did not consume at least 80 % of the mean food uptake per treatment group were excluded from the evaluation.

Statistical analysis was performed using ToxRat Professional, Version 3.2.1, ToxRat Solutions GmbH.

Analytical method

Samples of feeding solution were analysed using the validated analytical method M-704649-01-1, report reference M-704649-01-1 (**see Doc MCP Section 5**).

II. Results and Discussion

Validity criteria according to the OECD 246 and OECD 247 guideline (2017), to which the study was conducted, were met.

- Mortality in the water control should be ≤10% at test termination (actual: 3.3% in oral and contact tests)
- Mortality in the toxic reference group should be ≥50% at test termination (actual: 53.3% in the contact test; 100% in the oral test)

For the contact test, analysis of the highest application solution of 400 µg product/bumblebee gave recoveries of 97% and 96% for prothioconazole and spiroxamine, respectively. Analysis of the lowest application solution of 25 µg product/bumblebee gave recoveries of 95% and 95% for prothioconazole and spiroxamine, respectively.

For the oral test, analysis of the highest application solution of 320 µg product/bumblebee gave recoveries of 89% and 90% for prothioconazole and spiroxamine, respectively. Analysis of the lowest application solution of 20 µg product/bumblebee gave low recoveries of 47% and 42% for prothioconazole and spiroxamine, respectively. However, for the highest dose application solution of 320 µg product/bumblebee, at which there was no bumblebee mortality, the recoveries were approximately 90 % of the nominal concentrations thereby confirming the correct dosing of the bumblebees at the highest treatment of 200.8 µg product/bumblebee.

At termination of the contact test, exposure to 400, 200, 100, 50 and 25 µg product/bumblebee led to 10, 3.3, 0, 3.3 and 0% mortality, respectively. 3.3% mortality occurred in the water control treatment group and 53.3% mortality occurred in the reference item treatment group.

During the 4 hours assessment, one moribund bumblebee was observed in the 400, 200 and 50 µg product/bumblebee treatment groups, respectively. Also, one affected bumblebee was observed 48 hours after treatment in the 25 µg product/bumblebee treatment group. No test item induced behavioural effects were observed in the 100 µg product/bumblebee test item treated group.

Table CP 10.3.1.1.1/02-1 Summary of mortality and behavioural abnormalities observed during the contact test

Treatment group (µg product/ bumblebee)	4 hours		24 hours		48 hours	
	Mean mortality (%)	Mean behavioural abnormalities (%)	Mean mortality (%)	Mean behavioural abnormalities (%)	Mean mortality (%)	Mean behavioural abnormalities (%)
400	0	3.3	3.3	0	10	0
200	0	3.3	3.3	0	3.3	0
100	0	0	0	0	0	0
50	0	3.3	3.3	0	3.3	0
25	0	0	0	0	0	3.3
Water control	0	0	0	0	3.3	0
Dimethoate: 10 µg a.s./bumblebee	0	3.3	26.7	95.5	53.3	71.4

At termination of the oral test, achieved dose levels of 200.8, 139.4, 78.4, 40.4 and 22.6 µg product/bumblebee led to 0, 0, 0, 0, and 3.3% mortality, respectively. 3.3% mortality occurred also in the water control treatment group and 100% mortality occurred in the reference item treatment group.

During the 4 hours assessment, two affected bumblebees were observed in the 200.8 µg product/bumblebee test item treatment group. No test item induced behavioural effects were observed in the other test item treatment groups.

Table CP 10.3.1.1.1/02-2 Summary of mortality and behavioural abnormalities observed during the oral test

Treatment group (µg product/ bumblebee)	4 hours		24 hours		48 hours	
	Mean mortality (%)	Mean behavioural abnormalities (%)	Mean mortality (%)	Mean behavioural abnormalities (%)	Mean mortality (%)	Mean behavioural abnormalities (%)
200.8	0	20	0	0	0	0
139.4	0	0	0	0	0	0
78.4	0	0	0	0	0	0
40.4	0	0	0	0	0	0
22.6	0	0	0	0	3.3	0

Treatment group (µg product/ bumblebee)	4 hours		24 hours		48 hours	
	Mean mortality (%)	Mean behavioural abnormalities (%)	Mean mortality (%)	Mean behavioural abnormalities (%)	Mean mortality (%)	Mean behavioural abnormalities (%)
Water control	0	0	0	0	3.3	0
Dimethoate: 4.5	0	96	100	-	100	-

Table CP 10.3.1.1.1/02-3 LD_x and NOED values for bumblebees exposed to Prothioconazole + Spiroxamine EC460 in contact and oral tests

Parameter	Value (µg product/bumblebee)	
	24 hours	48 hours
Contact LD_{50}	>400	>400
Contact LD_{20}	>400	>400
Contact LD_{10}	>400	>412.5
Contact NOED	≥400	≥400
Oral LD_{50}	>200.8	>200.8
Oral LD_{20}	>200.8	>200.8
Oral LD_{10}	>200.8	>200.8
Oral NOED	≥200.8	≥200.8

III. Conclusion

Bumblebees were exposed to Prothioconazole + Spiroxamine EC 460 in a 48-hour oral and contact toxicity study.

In the contact test, following application of Prothioconazole + Spiroxamine EC 460, the 48-hour LD_{50} was considered to be >400 µg product/bumblebee. The 48-hour NOED value was ≥400 µg product/bumblebee.

In the oral test, following exposure to Prothioconazole + Spiroxamine EC 460, the 48-hour LD_{50} was considered to be >200.8 µg product/bumblebee. The 48-hour NOED value was ≥200.8 µg product/bumblebee.

Assessment and conclusion by applicant:

Validity criteria according to the OECD 246 and OECD 247 guideline (2017), to which the study was conducted, were met:

- Mortality in the water control should be ≤10% at test termination (actual: 3.3% in oral and contact tests)
- Mortality in the toxic reference group should be ≥50% at test termination (actual: 53.3% in the contact test; 100% in the oral test)

The low recovery of prothioconazole and spiroxamine in the oral test application solution at 20 µg product/bumblebee is noted. However, for the highest dose application solution of 320 µg product/bumblebee, at which there was no bumblebee mortality, the recoveries were approximately 90 % of the nominal concentrations for prothioconazole and spiroxamine thereby confirming the correct dosing of the bumblebees at the highest oral test treatment of 200.8 µg product/bumblebee (based on actual consumption). The determined LD_{50} of >200.8 µg product/bumblebee is therefore considered to be reliable and the low recovery at the lowest application solution has no impact on this result.

The study is therefore considered acceptable.

The 48-hour LD_{50} in the oral test was considered to be >200.8 µg product/bumblebee. The 48-hour LD_{50} in the contact test was considered to be >400 µg product/bumblebee.

CP 10.3.1.1.2 Acute contact toxicity to bees

Please refer to Section CP 10.3.1.1.1 for summaries of the available acute contact toxicity tests.

CP 10.3.1.2 Chronic toxicity to bees

Data Point:	KCP 10.3.1.2.01
Report Author:	[redacted]
Report Year:	2020
Report Title:	Prothioconazole + spiroxamine EC 460: Chronic oral toxicity test on the honey bee (*Apis mellifera* L.) in the laboratory
Report No:	143101136
Document No:	M-690829-01-1
Guideline(s) followed in study:	(Regulation (EC) No. 1107/2009 Directive 2003-01 (Canada/PMRA) US EPA OCSPP 850.SUPP. OECD Guideline 245 (2017)
Deviations from current test guideline:	None
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

Honeybees (*Apis mellifera* L.) were exposed to five concentrations of prothioconazole + spiroxamine EC 460 by *ad libitum* feeding over a period of 10 days. Test concentrations were 10000, 5000, 2500, 1250 and 625 mg product/kg feeding solution.

Mortality levels of 100%, 90%, 40% and 6.7% occurred in the concentration groups of 10000, 5000, 2500 and 1250 mg product/kg feeding solution (corresponding to a mean dietary dose of 126, 63.4, 31.6 and 21.1 µg a.s./bee/day), respectively, at test termination.

The NOEC was determined to be 888.1 mg product/kg feeding solution. The NOEDD was determined to be 15.0 µg a.s./bee/day.

The LC_{50} was determined to be 2003.9 mg product/kg feeding solution and the LDD_{50} was determined to be 26.8 µg a.s./bee/day.

I. Materials and Methods

Materials

Test Material	Prothioconazole + Spiroxamine EC 460
Lot/Batch #:	EM4L025614
Purity:	Prothioconazole: 16.2% w/w, 159.0 g/L Spiroxamine: 30.1% w/w, 259.7 g/L
Description:	Yellow brown liquid
Reanalysis/Expiry date:	08 April 2021
Density:	0.983 g/mL
Treatments	
Test rates:	Nominal: 10000, 5000, 2500, 1250 and 625 mg product/kg feeding solution equivalent to 200, 100, 50.0, 25 and 12.5 µg product/bee/day
Solvent/vehicle:	50% w/v sucrose solution
Analysis of test concentrations:	Yes. Analysis of feeding solutions on each day of the test
Test organisms	
Species:	*Apis mellifera* L., 2 days old from two queen-right colonies
Source:	In-house culture
Acclimatisation period:	At least one day
Feeding:	Fed *ad libitum* 50% (w/v) sucrose solution containing the test item, the reference item or untreated
Treatment for disease:	Not reported
Test design	
Test vessel:	Stainless steel cages (*ca.* 8 x 6 x 4 cm)
Replication:	3 replicates per test item dose level, controls and reference item dose
No. animals/vessel:	10 per test vessel
Duration of test:	10 days
Environmental test conditions	
Temperature:	31 – 33°C
Relative humidity:	50 – 70%
Photoperiod:	Darkness (except during observation)

Study Design

Young adult worker honey bees (2 days old at test initiation) from *Apis mellifera* L. were exposed to a control treatment, one reference item treatment and five concentrations of Prothioconazole + spiroxamine EC 460 by *ad libitum* feeding over a period of 10 days.

One brood comb with sealed brood from five hives in which bees were visibly starting to emerge were used in the test. These combs contained pollen which was used as a first feeding source for the freshly hatched bees. The combs were taken from the hive and adult bees were removed. The combs were transferred to the laboratory and placed into a hatching box. The box was placed into an incubator for one day to let the bees hatch under test conditions. The next day the hatched bees were collected and randomly assigned into cages (test units) in groups of 10 bees. The following day the test was initiated (Day 0, first dose administration) with 1-2 days old worker honey bees. Moribund bees were rejected and replaced by healthy bees prior to first feeding.

The bees were housed in cages made of stainless steel (*ca.* 8 x 6 x 4 cm) and incubated within 31 to 33ºC. Each treatment group consisted of 30 organisms (divided into 3 replicates, containing 10 test organisms each).

The control group were fed with untreated aqueous sucrose solution and the treatment groups were fed with sucrose solution containing the test item. Prothioconazole + Spiroxamine EC 460 was administered at nominal concentrations of 10000, 5000, 2500, 1250 and 325 mg product/kg feeding solution, equivalent to 200, 100, 50.0, 25 and 12.5 µg product/bee/day. The reference item group were exposed to 1 mg a.s./kg feeding solution of BAS 152 11 I (dimethoate).

The bees were fed *ad libitum* with a 50 % (w/v) sucrose solution containing the test item (test item group), the reference item (reference item group) or the sugar solution only (control group). The feeding solutions were provided in syringes and daily replaced by freshly prepared solutions.

In order to adjust for possible evaporation of test solutions from the feeders, 3 cages were set up containing pre-weighted syringes filled with sugar solution in absence of bees. The syringes were weighted and replaced daily. The evaporation figure was determined daily by weighting feeders from separate cages without honey bees. The measured difference was subtracted from the measured uptake to adjust the values for the loss by evaporation.

The daily food consumption per bee was calculated by the number of surviving bees per assessment and the amount of food consumed in the following assessment day.

Duplicate samples of the feeding solutions of the test item (5 concentrations) and control were taken for chemical analysis on day 0 + 9.

Mortality and behavioural abnormalities were recorded daily after application (start of feeding) during the 10-day exposure period. The chronic effects of Prothioconazole + spiroxamine EC 460 were evaluated by comparing the results of the test item group to those of the treatment groups. The software used to perform the statistical analysis was ToxRat Professional, Version 3.2.1, ® ToxRat Solutions GmbH.

Analytical method

Samples of feeding solution were analysed using the validated analytical method M-690829-01-1, report reference M-690829-01-1 (**see Doc MCP Section 5**).

II. Results and Discussion

Validity criteria according to the OECD 245 guideline (2017) were met.

- The average mortality across replicates for the untreated control should be ≤15% (actual: 0.0% on Day 10)
- The average mortality across replicates for the reference substance should be ≥50% (actual: 100% by Day 5)

The analytical recovery rates of the active substances prothioconazole and spiroxamine in the feeding solutions were within a range of 43 % to 108 % of the nominal value in case of prothioconazole and between 45 % and 123 % for spiroxamine, respectively. The results have been corrected for the analytical recovery rate.

When adjusted for analytical recovery the test concentrations were 9765.0, 3757.5, 1820.0, 888.1 and 445.0 mg product/kg feeding solution.

In the oral toxicity test actual consumed doses were determined to be 126.0, 63.4, 31.6, 21.1 and 11.0 µg product/bee/day. When adjusted for analytical recovery the actual consumed doses were 123.0, 47.6, 23.0, 15.0 and 7.8 µg product/bee/day.

Mortality levels of 100%, 90%, 40% and 6.7% occurred at nominal concentration groups of 10000, 5000, 2500 and 1250 mg product/kg feeding solution (corresponding to a mean dietary dose of 126, 63.4, 31.6 and 21.1 µg product/bee/day), respectively, at test termination.

No mortality occurred in the 625 mg product/kg feeding solution (corresponding to 11.0 µg product/bee/day) and untreated control group (50 % w/v sucrose solution) at test termination.

The reference item at a concentration of 1 mg a.s./kg feeding solution (corresponding to an actual mean dietary dose of 0.014 µg a.s./bee/day) caused a continuously increasing mortality leading to 100% mortality at day 5.

Table CP 10.3.1.2/01-1 Summary of mortality data following exposure to Prothioconazole + Spiroxamine EC 460

Treatment group (mg product/kg)	Mean mortality (%)									
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10
10000	10	30	53.3	73.3	83.3	93.3	96.7	100	100	100
5000	0	6.7	16.7	30	40	53.3	60	73.3	86.7	90
2500	0	0	0	3.3	6.7	13.3	13.3	26.7	33.3	40
1250	0	0	0	0	0	0	0	0	0	6.7
625	0	0	0	0	0	0	0	0	0	0
Reference item: 1.0	0	0	30	80	100	100	100	100	100	100
Control	0	0	0	0	0	0	0	0	0	0

From test initiation, behavioural abnormalities (*e.g.* moribund affected or apathetic bees) were observed in a time and concentration related manner in the treatment groups receiving concentrations levels of 10000, 5000 and 2500 mg product/kg feeding solution. No behavioural abnormalities occurred in the treatment groups provided with 1250 and 625 mg product/kg feeding solutions.

Table CP 10.3.1.2/01-2 Summary of behavioural abnormalities following exposure to Prothioconazole + Spiroxamine EC 460

Treatment group (mg product/kg)	Behavioural abnormalities (%)									
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10
10000	10	0	0	0	0	0	0	0	0	0
5000	3.3	6.7	0	3.3	0	0	0	3.3	0	0
2500	3.3	0	6.7	0	0	0	0	0	0	0
1250	0	0	0	0	0	0	0	0	0	0

Treatment group (mg product/kg)	Behavioural abnormalities (%)									
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10
625	0	0	0	0	0	0	0	0	0	0
Reference item: 1.0	0	0	13.3	3.3	0	0	0	0	0	0
Control	0	0	0	0	0	0	0	0	0	0

Table CP 10.3.1.2/01-3 Summary of mortality and endpoints following exposure to Prothioconazole + Spiroxamine EC 460

Test Object		*Apis mellifera carnica*	
Treatment Group	Concentration [mg product/kg feeding solution]	Dietary Dose [1] [µg product/bee/day]	Mortality at day 10 [2], [% Mean]
Prothioconazole + Spiroxamine EC 460	10000 (9765.0)[3]	126.0 (123.0)[4]	100.0 (*)
	5000 (3757.5)[3]	63.4 (47.6)[4]	90.0 (*)
	2500 (1820.0)[3]	34.6 (23.0)[4]	40.0 (*)
	1250 (888.1)[3]	21.1 (15.0)[4]	6.7 (n.s.)
	625 (445.0)[3]	10.0 (7.8)[4]	0.0 (n.s.)
Water control			0.0
Reference Item	1.0	0.014	100.0

Endpoint at test termination (day 10)			
LC_{50}	LDD_{50}	LC_{20}	LDD_{20}
2003.9 mg product/kg feeding solution	26.8 µg product/bee/day	1310.5 mg product/kg feeding solution	18.8 µg product/bee/day
LC_{10}	LDD_{10}	NOEC	NOEDD
1049.6 mg product/kg feeding solution	15.6 µg product/bee/day	888.1 mg product/kg feeding solution	15.0 µg product/bee/day

[1] mean dose per bee per day; dose measured based on consumed feeding solution
[2] Mortality at study termination 10 days after start of first feeding
[3] Values in parentheses were corrected dietary concentrations based on the mean values of the dose verification
[4] Values in parentheses were corrected dietary doses based on the mean values of the dose verification
Dietary concentrations and doses of the tets item were corrected by mean dose verification values of prothioconazole and spiroxamine each treatment group (see table in page 12).

Statistics: all values were based on the analytical corrected concentrations or dose levels.
$LC_{10/20/50}$/$LDD_{10/20/50}$: were determined according to Probit Analysis (according to Finney 1971)
NOEC/NOEDD: were determined using Fisher's Exact Binomial Test (one-sided greater, $\alpha = 0.05$)
n.s. = no statistical significant difference compared to the control, * = statistically significant different compared to the control ($\alpha = 0.05$)

III. Conclusion

Adult honeybees (*Apis mellifera* L.) were exposed to Prothioconazole + spiroxamine EC 460 in a 10-day chronic feeding test.

After 10 days exposure, the NOEC was determined to be 888.1 mg product/kg feeding solution. The NOEDD was determined to be 15.0 µg product/bee/day.

The LC_{50} was determined to be 2003.9 mg product/kg feeding solution and the LDD_{50} was determined to be 26.8 µg product/bee/day.

Assessment and conclusion by applicant:

Validity criteria according to the OECD 245 guideline (2017), to which the study was conducted, were met.

- The average mortality across replicates for the untreated control should be ≤15% (actual: 0.0% on Day 10)
- The average mortality across replicates for the reference substance should be ≥50% (actual: 100% by Day 5)

The study is therefore considered acceptable.

The LC_{50} was determined to be 2003.9 mg product/kg feeding solution which is equivalent to 325 mg prothioconazole/kg feeding solution and 603 mg spiroxamine/kg feeding solution.

The LDD_{50} was determined to be 26.8 µg product/bee/day which is equivalent to 4.34 µg prothioconazole/bee/day and 8.07 µg spiroxamine/bee/day. The NOEDD was determined to be 15.0 µg product/bee/day.

Data Point:	KCP 10.3.1.2/02
Report Author:	[redacted]
Report Year:	2020
Report Title:	Prothioconazole + spiroxamine EC 460 (160+300 g/L): Chronic toxicity to the honey bee Apis mellifera L. under laboratory conditions - Final report
Report No:	19 48 BAC 0019
Document No:	M-755308-01-1
Guideline(s) followed in study:	EU Directive 91/414/EEC Regulation (EC) No 1107/2009 (2009) US EPA OCSPP 850.SUPP OECD 245 (adopted 9 October 2017)
Deviations from current test guideline:	None
Previous evaluation:	No, not previously submitted -
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

Honeybees (*Apis mellifera* L.) were exposed to five concentrations of Prothioconazole + Spiroxamine EC 460 (160+300 g/L) in a 10 day chronic oral toxicity test. A toxic reference concentration was also tested.

The NOEDD was determined to be 9.52 µg product/bee/day and the NOEC to be 386 mg product/kg food.

Since the obtained mortalities did not reach 50% the LDD_{50} is considered to be higher than 13.9 µg product/bee/day and the LC_{50} to be higher than 672 mg product/kg food.

I. Materials and Methods

Materials

Test Material	Prothioconazole + spiroxamine EC 460 (160+300 g/L)
Lot/Batch #:	EM4L025614
Purity:	Content of active ingredients analysed: Prothioconazole 16.2% w/w corresponding to 159.0 g/L Spiroxamine 30.1% w/w corresponding to 295.7 g/L
Description:	Liquid, clear, yellow brown
Stability of test compound:	Not reported
Reanalysis/Expiry date:	08 April 2021
Density:	0.983 g/mL
Treatments	
Test rates:	3.14, 6.27, 12.5, 25.1 and 50.2 mg a.s./bee/day
Solvent/vehicle:	Sucrose solution
Analysis of test concentrations:	The mean recovery of prothioconazole was between 44.4% and 85.3% and the mean recovery of spiroxamine was between 60.9% and 88.7%. The theoretically consumed doses and nominal concentrations were corrected for the mean recoveries.
Test organisms	
Species:	*Honey bee, Apis mellifera L.* subspecies Buckfast (Hymenoptera, Apoidea), colonies in good health condition, well fed and queen-right
Source:	On-site stock
Acclimatisation period:	24 ± 2 hours in test cages at 33 ± 2°C and 50 – 70% RH
Feeding:	*Ad libitum* 50% (w/v) sucrose solution
Treatment for disease:	All bees used in the test derived from healthy, disease free and queen-right bee colonies
Test design	
Test vessel:	Aluminium cages with the dimensions: 95 mm (width) ×70 mm (height) × 60 mm (depth); with holes in the lateral walls for ventilation and two glass plates (one in front and one in the back) for observations of the bees.
Replication:	Triplicate
No. animals/vessel:	10

Duration of test:	10 days
Environmental test conditions	
Temperature:	32.0 – 33.6°C
Relative humidity:	55.4 – 62.1%
Photoperiod:	24-hour darkness

Study Design

The exposure took place for a period of 10 days. Test item solutions were prepared daily just before administration of food. The reference item stock solution was prepared once for the whole feeding period and stored in the refrigerator at about 6 °C (the reference item Dimethoate is stable over a period of 10 days when stored in the refrigerator). The reference item feeding solution was prepared at least every 4 days and stored in the refrigerator at about 6 °C. The daily dose rates (administered solution) were based on a theoretical oral consumption of 33 µL per bee and day, which is described in literature.

Young worker honeybees (newly hatched; 1 to 2 days old) from *Apis mellifera* L. were exposed to a control treatments, one reference item treatment and five concentrations of Prothioconazole + spiroxamine EC 460 (160+300 g/L) by continuous and *ad libitum* feeding over a period of 10 days.

The bees were housed in aluminium cages with the dimensions: 95 mm (width) × 70 mm (height) × 60 mm (depth) and kept between 32.0 to 33.6°C. Each treatment group consisted of 30 test organisms (divided into three replicates, containing 10 test organisms each). The control group was fed with untreated aqueous sucrose solution.

Prothioconazole + spiroxamine EC 460 (160+300 g/L) was administered at concentrations of 3.14, 6.27, 12.5, 25.1 and 50.2 µg a.s./bee/day, corresponding to concentrations of 1277, 639, 319, 160 and 80 mg product/kg food, respectively. The reference item group were exposed to 27.3 ng a.s./bee/day of BAS 152 11 I (dimethoate EC 400).

The bees were fed with 50% (w/v) aqueous sucrose solution including the test item or the reference item. The control group was fed with 50% (w/v) aqueous sucrose solution. The treated/untreated food was provided *ad libitum* in a plastic syringe, which had been weighed before addition to the test chambers. The feeders remained in the cages for about 24 h (± 2 h). The actual consumption was determined by reweighing the syringe containing the remaining test solution each day after removal from the test units. Any unconsumed food was rejected.

The evaporation of test solution from the feeders was investigated in additional test cages which were set up with the main test. These cages contained no bees, only pre-weighed feeders containing diet of untreated control and were placed in the test environment alongside the test units. At the daily feeder exchange the feeders were re-weighed and replaced by new feeders. This evaporation figure was subtracted from the calculated food consumption to give the corrected food consumption accounting for the loss by evaporation.

Mortality was recorded daily at about the same time of the day (every 24 h ± 2 h), starting 24 ± 2 hours after start of the test period (initial feeding). Additionally, behavioural abnormalities were recorded daily at the same time as the assessments of mortality according to the following categories: healthy/normal, moribund (M), affected in terms of uncoordinated movements (A), cramping (C), apathetic/lethargy (Ap), omiting/regurgitation (V). Any other behavioural abnormalities were noted and clearly described, if observed.

For verification of the exposure concentration, all test item solutions (AT, BT, CT, DT and ET) and the control solution (AC) were sampled in duplicate as specimens for analysis and retained samples directly after preparation on each day of application.

The statistical calculations were performed with the computer program ToxRat Professional 3.3.0 (2018). Step-down Cochran-Armitage Test Procedure was used for mortality data (one-sided greater; α = 0.05) and determination of NOEDD/NOEC (no observed effect dietary dose/ concentration). Mortality data were arcsine-transformed for determination of LDDX/LCX (lethal dietary doses/ concentrations) by Weibull analysis using linear max. likelihood regression.

Analytical method

Samples of feeding solution were analysed using the validated analytical method M-687692-01-1, report reference M-687692-01-1 (**see Doc MCP Section 5**).

II. Results and Discussion

Validity criteria according to the OECD 245 guideline (2017) were met.

- Mortality in the control group should be ≤15%: 0.0% mean mortality in control group AC after 10 days of exposure
- Mortality in the reference group should be ≥50% - 100% mean mortality after 10 days of exposure

The concentrations of the active substances prothioconazole and spiroxamine were determined in feeding solutions of all treatment groups. The mean recovery of prothioconazole was between 44.4% and 85.3% and the mean recovery of spiroxamine was between 60.9% and 88.7%. The theoretical consumed doses and nominal concentrations were corrected for the mean recoveries. Neither prothioconazole nor spiroxamine were detected in the control samples.

In the chronic toxicity feeding test a mean mortality of 0.0% was observed in control group AC after 10 days.

Based on the actual consumption of feeding solution the effective doses were 26.4, 15.8, 8.90, 4.92 and 2.64 µg product/bee/day which resulted in mortalities of 30.0, 3.3, 0.0, 3.3 and 0.0%, respectively after 10 days. Due to lower recovery rates the concentrations and doses were corrected for the actual rates resulting in doses of 13.9, 9.52, 6.21, 4.17 and 2.30 µg product/bee/day, corresponding to concentrations of 672, 386, 223, 135 and 69 mg product/kg food, respectively. The obtained mortality in the 13.9 µg product/bee/day treatment group was statistically significantly increased compared to the control group AC.

The reference dosage tested in the study was 27.3 ng a.i./bee/day (actual consumption on average per day: 20.9 ng a.i./bee), which caused a mean mortality of 100.0%).

In the test item group the food consumption ranged between 20.6 and 33.1 mg solution per bee per day (control AC: on average 35.8 mg/bee/day) with a tendency of higher food uptake in the lower test item doses. The food consumption per cage was corrected by subtracting the mean evaporation figure of each day of application.

There were no behavioural abnormalities in any of the treatment groups observed during the entire test period.

Table CP 10.3.1.2/02-1 Mesured concentrations in feeding solutions

Treatment group ID	Nominal concentration (mg a.s./kg food)	Measured concentration (mg a.s./kg food)	Mean recovery (%)
Prothioconazole			
AT	207	91.7	44.4
BT	103	58.0	56.2
CT	52	37.7	73.0
DT	26	21.8	84.3

ET	13	11.0	85.3
Spiroxamine			
AT	384	234	52.6
BT	192	124	60.4
CT	96	63.9	69.8
DT	48	41.0	84.8
ET	24	21.3	87.0

Mean recovery rates over 10 days

Table CP 10.3.1.2/02-2 Mean cumulative mortality (%) in the course of the study

Treatment group		Control	Test item					Reference item		
Nominal daily dose (µg product/bee/day)		AC -	AT 50.2	BT 25.1	CT 12.5	DT 6.27	ET 3.14	Nominal daily dose (µg a.s./bee/day)		AR 27.3
Mean cumulative mortality (%)	D1	0.0	0.0	0.0	0.0	0.0	0.0	Mean cumulative mortality (%)	D1	0.0
	D2	0.0	0.0	0.0	0.0	0.0	0.0		D2	6.7
	D3	0.0	6.7	0.0	0.0	0.0	0.0		D3	26.7
	D4	0.0	6.7	0.0	0.0	0.0	0.0		D4	73.3
	D5	0.0	6.7	0.0	0.0	3.3	0.0		D5	86.7
	D6	0.0	10.0	0.0	0.0	3.3	0.0		D6	100.0
	D7	0.0	10.0	0.0	0.0	3.3	0.0		D7	100.0
	D8	0.0	13.3	0.0	0.0	3.3	0.0		D8	100.0
	D9	0.0	20.0	0.0	0.0	3.3	0.0		D9	100.0
	D10	0.0	30.0	3.3	0.0	3.3	0.0		D10	100.0

Calculations are performed with non-rounded values

D = Day

Table CP 10.3.1.2/02-3 Study endpoints

	Endpoints	Value
Test item doses	LDD_{50} [µg consumed product/bee/day]	>13.9
	LDD_{20} [µg consumed product/bee/day]	10.7 (7.82 – 19.4)
	LDD_{10} [µg consumed product/bee/day]	5.58 (3.20 – 7.61)
	NOEDD [µg consumed product/bee/day]	9.52
Test item concentrations	LC_{50} [mg product/kg food]	>672
	LC_{20} [mg product/kg food]	456 (314 – 885)
	LC_{10} [mg product/kg food]	203 (108 – 296)
	NOEC [mg product/kg food]	386

Values in parentheses are 95% confidence limits

III. Conclusion

Since the obtained mortalities did not reach 50% the LDD_{50} is considered to be higher than 13.9 µg product/bee/day and the LC_{50} to be higher than 672 mg product/kg food.

The LDD_{20} was determined to be 10.7 µg product/bee/day and the LC_{20} to be 456 mg product/kg food.

The LDD_{10} was determined to be 5.58 µg product/bee/day and the LC_{10} to be 203 mg product/kg food.

The NOEDD was determined to be 9.52 µg product/bee/day and the NOEC to be 386 mg product/kg food.

Assessment and conclusion by applicant:

Validity criteria according to the OECD 245 guideline (2017), to which the study was conducted, were met.

- Mortality in the control group should be ≤15%: 0.0% mean mortality in control group AC after 10 days of exposure
- Mortality in the reference group should be ≥50%: 100% mean mortality after 10 days of exposure

The study is therefore considered acceptable. The results have been corrected for the recovery of the active substances in the feeding solutions.

The LDD_{50} is considered to >13.9 µg product/bee/day and the LC_{50} to be >672 mg product/kg food. The NOEDD was determined to be 9.52 µg product/bee/day.

Data Point:	KCP 10.3.1.2/03
Report Author:	[redacted]; [redacted]
Report Year:	2020
Report Title:	Analytical phase report - Prothioconazole + spiroxamine EC 460 (160+300 g/L): Chronic toxicity to the honey bee Apis mellifera L. under laboratory conditions
Report No:	19 48 BAC 0019-P1
Document No:	M-687692-01-1
Guideline(s) followed in study:	Regulation (EC) No 1107/2009 of the European Parliament and the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC [2] European Commission Guidance Document for Generating and Reporting Methods of Analysis in Support of Pre-Registration Data Requirements for Annex II (Part A, Section 4) and Annex III (Part A, Section 5) of Directive 91/414, SANCO/3029/99 rev. 4, 11/07/00 [3] Guidance document on residue analytical methods, SANCO/825/00 rev. 8.1, European Commission, Directorate General Health and Consumer Protection 16/11/2010 [4] US EPA Residue Chemistry Guideline OCSPP 860.1340: Residue Analytical Method [5]
Deviations from current test guideline:	None
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The purpose of this study was to determine the chronic oral toxicity of the test item prothioconazole + spiroxamine EC 460 (160+300 g/L) to adult worker bees of *Apis mellifera* L, under laboratory conditions. The objective of the analytical phase of the study was to analyse the test item treated 50% (w/v) aqueous sucrose solution of each test item group and the control group for residues of prothioconazole and spiroxamine for verification of test item concentration.

The mean recovery values per fortification level of prothioconazole in sugar solution ranged between 92 and 102% with relative standard deviations between 1.7 and 5.6%. The overall mean recovery was 96% and the corresponding overall relative standard deviation (RSD) was 5.2% (n = 12).

The mean recovery values per fortification level of spiroxamine in sugar solution ranged between 73 and 95% with relative standard deviations between 1.6 and 3.9%. The overall mean recovery was 89% and the corresponding overall relative standard deviation (RSD) was 11.1% (n = 12).

All results of the method validations were in accordance with the general requirements for residue analytical methods; therefore, the employed method was validated successfully.

I. Analytical method

Residues of prothioconazole and spiroxamine in/on bee relevant matrices were determined by HPLC-MS/MS according to method 01013/M001. The residues from bee relevant matrices were diluted with a mixture of acetonitrile/water. Afterwards, the stable isotopically labeled analytes were added. The solution was made up to volume, diluted where necessary and subjected to reversed phase HPLC-MS/MS. Prothioconazole was detected in the negative (ESI-) ion mode and spiroxamine was detected in the positive (ESI+) ion mode. Residues were quantified using internal stable labeled standards.

Sample preparation

A representative aliquot of 1 g of the sample was weighed into a 50 mL falcon tube, for recovery experiments fortify the weighed sample with the corresponding amount of test item and add 25 mL of acetonitrile/water (1/1; v/v) and 2 mL of a 250 g/L cysteine hydrochloride solution. The sample was placed on the overhead shaker and the sample were shaken until the whole sample is solved. 0.05 mL of an internal standard solution containing 1000 µg/L was added and the solution was well shaken. The sample was transferred into a 50 mL volumetric flask and filled up to the final volume with acetonitrile/water (1/1; v/v). An aliquot of the sample solution was centrifuged at 13000 rpm for 5 minutes and subjected to HPLC-MS/MS determination. Samples containing high analyte concentrations were diluted until their concentrations were within the linearity range of the corresponding calibration curve.

For the feeding diets concurrent recovery determinations were included in each set of analyses (at least one recovery for ten study samples). The respective Limit of Quantification (LOQ) of prothioconazole and spiroxamine, defined as the lowest validated fortification level, was 0.01 mg/kg in feeding diets. The corresponding respective Limit of Detection (LOD) was 0.003 mg/kg.

HPLC-Instrument Conditions

An aliquot of the prepared sample was injected into the High-Performance Liquid Chromatograph (HPLC), chromatographed under gradient reversed phase conditions and detected by Tandem Mass Spectrometry (MS/MS) with electrospray ionization (ESI).

Mass Spectrometry

The detection by MS/MS was performed on a triple-quadrupole tandem mass spectrometer, equipped with a Turbo Ion Spray (ESI) interface operated in the positive and negative ion mode and multiple reaction monitoring mode (MRM). Unit mass resolution was established in the mass resolving quadrupoles by maintaining a full width at half-maximum (FWHM) of about 0.7 amu (= Unit Mass Resolution). Optimal collision-activated dissociation (CAD) conditions for fragmentation of the pseudomolecular ions of the analytes were applied with nitrogen as the collision gas.

Method Performance

Full validation data is documented within the method 01013/M001 itself for plant sample material. For the feeding diet a limited set of validation recoveries (one control sample, at least 3 repetitions each at two fortification levels) at the LOQ (0.01 mg/kg) and at the 10-fold LOQ level (0.10 mg/kg) was performed within this study. In order to check the performance of the method, concurrent recovery determinations were included in each set of analysis (at least one recovery for ten study samples). Additional concurrent recoveries were performed at 1000 x LOQ (3 x 10 mg/kg) for prothioconazole and 50000 x LOQ (3 x 500 mg/kg) for spiroxamine, respectively.

All results of the method validation were in accordance with the general requirements for residue analytical methods; therefore, the method was validated successfully.

Calculation of the residues

For calculation of the concentrations, calibration curves were used. These curves were calculated automatically after each sequence run with the Sciex quantitation software Analyst or LIMS.

The following equation was used for calculation of amounts using the internal standard procedure for a linear calibration curve of type y = ax + b:

$$C_S = \frac{\text{Area Ratio - Intercept (b)}}{\text{Slope (a)}} \cdot \frac{V_{End}}{M_S} \cdot DF$$

CS	Concentration in the sample [mg/kg]
Area Ratio	Peak area of the analyte in the sample solution [area counts], divided by the Peak area of the

internal standard in the sample solution [area counts]

Intercept Point where the calibration line crosses the y-axis [area counts/area counts]

Slope Slope of the calibration line [(area counts/area counts) / (µg/L)]

=> (1/(µg/L) => L/µg (in this case IS Conc. is set to 1)

V_{End} Final volume of the extract [L]

M_s Mass of the extracted sample [g]

DF Optional Dilution Factor, depending on possible dilutions of the sample. Aliquots and final volumes [mL] have to be considered. If the sample was not diluted this value is 1

II. Analytical Results and Discussion

All residues in control samples from the diet solutions were below the LOD. Residues in the control and treated samples of feeding diets are shown in the following tables:

Table CP 10.3.1.2/03-1 Summary of prothioconazole in feeding diet

Sample-ID 1948BAC0019-	Actual conc. of prothioconazole (mg/kg)	Target conc. of prothioconazole (mg/kg)	Recovery from target (%)
D0-AC-AE	-	< LOD	-
D0-AT-AE	82.6	207	40
D0-BT-AE	50.1	103	49
D0-CT-AE	33.8	52	65
D0-DT-AE	19.8	26	76
D0-ET-AE	9.69	13	75
D1-AC-AE	-	< LOD	-
D1-AT-AE	82.6	207	40
D1-BT-AE	57.6	103	56
D1-CT-AE	40.9	52	79
D1-DT-AE	22.4	26	86
D1-ET-AE	11.9	13	92
D2-AC-AE	-	< LOD	-
D2-AT-AE	96.6	207	47
D2-BT-AE	57.7	103	56
D2-CT-AE	36.1	52	69
D2-DT-AE	21.9	26	84
D2-ET-AE	12.1	13	93
D3-AC-AE	-	< LOD	-

D3-AT-AE	94.2	207	46
D3-BT-AE	54.9	103	53
D3-CT-AE	39.6	52	76
D3-DT-AE	23.0	26	88
D3-ET-AE	11.2	13	86
D4-AC-AE	-	< LOD	-
D4-AT-AE	94.5	207	46
D4-BT-AE	52.4	103	51
D4-CT-AE	34.3	52	66
D4-DT-AE	21.1	26	81
D4-ET-AE	10.9	13	84
D5-AC-AE	-	< LOD	-
D5-AT-AE	80.8	207	39
D5-BT-AE	52.0	103	50
D5-CT-AE	29.2	52	56
D5-DT-AE	19.7	26	76
D5-ET-AE	10.3	13	79
D6-AC-AE	-	< LOD	-
D6-AT-AE	85.9	207	41
D6-BT-AE	54.9	103	53
D6-CT-AE	38.3	52	74
D6-DT-AE	21.8	26	84
D6-ET-AE	10.9	13	84
D7-AC-AE	-	< LOD	-
D7-AT-AE	85.7	207	41
D7-BT-AE	64.8	103	63
D7-CT-AE	36.3	52	70
D7-DT-AE	20.8	26	80
D7-ET-AE	10.4	13	80
D8-AC-AE	-	< LOD	-
D8-AT-AE	93.1	207	45

D8-BT-AE	62.8	103	61
D8-CT-AE	40.0	52	77
D8-DT-AE	22.1	26	85
D8-ET-AE	11.2	13	86
D9-AC-AE	-	< LOD	-
D9-AT-AE	121	207	58
D9-BT-AE	73.5	103	71
D9-CT-AE	48.7	52	94
D9-DT-AE	25.0	26	96
D9-ET-AE	11.6	13	89

Table CP 10.3.1.2/03-2 Summary of spiroxamine in feeding diet

Sample-ID 1948BAC0019-	Actual conc. of protioconazole (mg/kg)	Target conc. of protioconazole (mg/kg)	Recovery from target (%)
D0-AC-AE	-	< LOD	-
D0-AT-AE	148	384	39
D0-BT-AE	120	192	63
D0-CT-AE	52.8	96	55
D0-DT-AE	36.4	48	76
D0-ET-AE	19.2	24	80
D1-AC-AE	-	< LOD	-
D1-AT-AE	222	384	58
D1-BT-AE	126	192	66
D1-CT-AE	669	96	69
D1-DT-AE	42.2	48	88
D1-ET-AE	22.0	24	92
D2-AC-AE	-	< LOD	-
D2-AT-AE	266	384	69
D2-BT-AE	124	192	65
D2-CT-AE	69.0	96	72
D2-DT-AE	43.2	48	90

D2-ET-AE	23.4	24	98
D3-AC-AE	-	< LOD	-
D3-AT-AE	254	384	66
D3-BT-AE	129	192	67
D3-CT-AE	66.4	96	69
D3-DT-AE	42.9	48	89
D3-ET-AE	21.6	24	90
D4-AC-AE	-	< LOD	-
D4-AT-AE	241	384	63
D4-BT-AE	125	192	65
D4-CT-AE	63.6	96	66
D4-DT-AE	40.8	48	85
D4-ET-AE	20.6	24	86
D5-AC-AE	-	< LOD	-
D5-AT-AE	233	384	66
D5-BT-AE	118	192	68
D5-CT-AE	59.9	96	67
D5-DT-AE	38.0	48	83
D5-ET-AE	19.3	24	86
D6-AC-AE	-	< LOD	-
D6-AT-AE	252	384	66
D6-BT-AE	131	192	68
D6-CT-AE	64.9	96	67
D6-DT-AE	39.9	48	83
D6-ET-AE	20.6	24	86
D7-AC-AE	-	< LOD	-
D7-AT-AE	245	384	64
D7-BT-AE	126	192	66
D7-CT-AE	62.2	96	65
D7-DT-AE	39.8	48	83
D7-ET-AE	21.4	24	89

D8-AC-AE	-	< LOD	-
D8-AT-AE	208	384	54
D8-BT-AE	119	192	62
D8-CT-AE	65.2	96	68
D8-DT-AE	43.1	48	90
D8-ET-AE	21.9	24	91
D9-AC-AE	-	< LOD	-
D9-AT-AE	270	384	70
D9-BT-AE	122	192	64
D9-CT-AE	68.2	96	71
D9-DT-AE	43.8	48	91
D9-ET-AE	22.9	24	95

III. Evaluation and Discussion

The method validation was done with a set of recoveries at the LOQ (3 x 0.01 mg/kg, each) and 10 x LOQ (3 x 0.10 mg/kg, each) level. Additional concurrent recoveries were performed at 1000 x LOQ (3 x 10 mg/kg), each and 50000 x LOQ (3 x 500 mg/kg) for prothioconazole and 46000 x LOQ (3 x 460 mg/kg) for spiroxamine, respectively.

The mean recovery values per fortification level of prothioconazole in sugar solution ranged between 92 and 102% with relative standard deviations between 1.7 and 5.6%. The overall mean recovery was 96% and the corresponding overall relative standard deviation (RSD) was 5.2% (n = 12).

The mean recovery values per fortification level of spiroxamine in sugar solution ranged between 73 and 95% with relative standard deviations between 1.6 and 3.9%. The overall mean recovery was 89% and the corresponding overall relative standard deviation (RSD) was 11.1% (n = 12).

No residues of prothioconazole and spiroxamine above the LOD were found in any of the control bee feeding diets.

Assessment and conclusion by applicant:

The study report presents the analytical method and the results of the analysis for the 10-day oral toxicity study with honeybees (M-755308-01-1). As such the study is considered to be acceptable.

Please refer to the Analytical Methods section of the dossier for a full assessment of the analytical method used.

CP 10.3.1.3 Effects on honey bee development and other honey bee life stages

Data Point:	KCP 10.3.1.3/01
Report Author:	[redacted]
Report Year:	2020
Report Title:	Prothioconazole + spiroxamine EC 460 (160+300 g/L) - Repeated exposure to honey bee larvae (Apis mellifera L.) under laboratory conditions
Report No:	19 48 BLC 0024
Document No:	M-756625-01-1
Guideline(s) followed in study:	Regulation (EC) No 1107/2009 (2009) Directive 2003-01 (CANADA/PMRA) US EPA OCSPP 850.SUPP OECD Guidance Document 239 (2016)
Deviations from current test guideline:	No temperature data between days 1 and 3 as probe was not placed in test chamber, report noted this had no impact on the outcome of the study
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The purpose of this study was to determine the chronic toxicity of the test item to the honey bee larvae, *Apis mellifera* L., in an *in vitro* test after repeated exposure.

For the control, five test concentrations and reference item groups three replicate test vessels were prepared each housing 12 larvae.

In a 22-day repeated exposure larval toxicity study performed in a dose-response design with Prothioconazole + Spiroxamine EC 460 (160+300 g/L), the NOED and LOED was determined to be 48 µg and 120 µg product/larva (based on adult emergence), respectively. The NOEC and LOEC were 303 mg and 758 mg product/kg food, respectively.

The ED_{50}, ED_{20} and ED_{10} values (based on adult emergence) were determined to be 151.6 µg, 99.0 µg and 74.6 µg product/larva, respectively. The EC_{50}, EC_{20} and EC_{10} values were determined to be 958 mg, 625 mg and 471 mg product/kg food, respectively.

I. Materials and Methods

Materials

Test Material Prothioconazole + spiroxamine EC 460 (160+300 g/L)

Lot/Batch #: EM4L025614

Purity: Content of active ingredients analysed:
Prothioconazole 16.2% w/w corresponding to 159.0 g/L
Spiroxamine 30.1% w/w corresponding to 295.7 g/L

Description: Liquid, clear, yellow brown

Stability of test compound: Not reported

Reanalysis/Expiry date: 08 April 2021

Density: 0.983 g/mL

Treatments

Test rates:	7.7, 19.2, 48, 120 and 300 µg product/larvae
Solvent/vehicle:	Aqueous solution containing royal jelly, yeast, fructose and glucose
Analysis of test concentrations:	The mean recovery of prothioconazole and spiroxamine ranged between 83% and 109% in the final diets

Test organisms

Species:	Honey bee larvae (Hymenoptera, Apoidea), *Apis mellifera* L. subspecies Buckfast, commonly known as Buckfast bees
Source:	The colonies were maintained by BioChem agrar, Germany. All larvae used in the test derived from healthy (free of clinical symptoms of any disease) and queen-right bee colonies. The larvae were taken from hives that had not received treatments with chemical substances for at least one month
Acclimatisation period:	The bee colonies used for this test contained brood in all stages (eggs, larvae and pupae), and sufficient pollen and nectar stores
Feeding:	50% weight of fresh royal jelly and 50% weight of an aqueous solution containing varying amounts of yeast extract, glucose and fructose
Treatment for disease:	The larvae were taken from hives that had not received treatments with chemical substances for at least one month

Test design

Test vessel:	Crystal polystyrene grafting cells (CNE Nicotplast, internal diameter 9 mm) were placed in 48 well plates. Well plates were placed on an adjustable warming plate
Replication:	Triplicate
No. animals/vessel:	12
Duration of test:	22 days

Environmental test conditions

Temperature:	34.0 – 35.0°C
Relative humidity:	D3 – D8: 95 – 100%, D8 – D15: 78 – 82% and D15 – D22: 56 – 70%
Photoperiod:	Constant darkness throughout the test (diffuse artificial light only during handling and assessments).

Study Design

First instar honeybee larvae (newly hatched; 1 day old) from *Apis mellifera* L. were exposed to a control treatment, one reference item treatment and five concentrations of Prothioconazole + Spiroxamine EC 460 (160+300 g/L) by continuous and *ad libitum* feeding over a period of 22 days.

Prothioconazole + Spiroxamine EC 460 (160+300 g/L) was administered at concentrations of 7.7, 19.2, 48, 120 and 300 µg product/larva. The reference item group were exposed to 7.6 µg a.s./larva feeding solution of Dimethoate tech.

Three colonies were used in the test. Each of the three colonies was treated equally: On day -3 (D2-3) the respective queen of the colony was caged on an empty brood comb, which was fitted in an excluder

cage and thereafter placed in the hive. The queen laid her eggs solely on this comb. The caging time was approx. 30 h. In the afternoon of day -2 (D-2) the queen was released from the excluder. The comb was checked for the presence of freshly laid eggs, and kept confined with the excluder to avoid any additional egg laying. The selected comb was placed near to frames containing open brood in the hive. The eggs were incubated within the hive between day -2 (D-2) and day 1 (D1).

The larval diet was prepared with deionised, autoclaved water using the following ingredients:

- Diet A (fed to bees on Day 1): 50% weight of fresh royal jelly + 50% weight of an aqueous solution containing 2% weight of yeast extract, 12% weight of glucose and 12% weight of fructose
- Diet B (fed to bees on Day 3): 50% weight of fresh royal jelly + 50% weight of an aqueous solution containing 3% weight of yeast extract, 15% weight of glucose and 15% weight of fructose
- Diet C (fed to bees on Days 4, 5 and 6): 50% weight of fresh royal jelly + 50% weight of an aqueous solution containing 4% weight of yeast extract, 18% weight of glucose and 18% weight of fructose

To ensure a homogenous distribution of the test item within the larval food, the final diets were placed on a multitube vortex shaker at 2500 rpm for 5 minutes at room temperature. In order to eliminate small, stable bubbles that potentially could affect the uptake of feeding solution by the larvae, the final diets were shortly centrifuged at 3000 rpm for 20 seconds. Then, the final test item feeding solutions were heated up in a water bath set to 34.5 °C for about 30 min. Before feeding, the final diets were vigorously shaken on a vortex shaker in order to eliminate probable fractionation of the food components and to keep the test item homogeneously distributed.

The bees were housed in crystal polystyrene grafting cells (CNE Nicotplast, internal diameter 9 mm) were placed in 48 well plates. Well plates were placed on an adjustable warming plate. On day 1 (D1), untreated artificial diet was pipetted into the grafting cells, followed by the transfer of one larva per cell.

Before application, all conspicuously sick or dead larvae were swapped with apparently healthy individuals originating from the respective colony. All plates used in the study were randomised using a scheme, which is part of the raw data. Thereafter the plates were marked with study number, treatment group and replicate number.

Each treatment group consisted of 36 test organisms (divided into three replicates, containing 12 test organisms each). The control group was fed with untreated aqueous sucrose solution.

All final diets were sampled in duplicate as analysis and retain samples after preparation on D3, D4, D5 and D6.

Mortality (an immobile larva, one which did not react to contact stimulus, or a larva that did not consume all the diet by D8 was noted as dead), was recorded on day eight, daily observations on day four to day eight (larvae), and on day 15 (pupae). Adult emergence was recorded on day 22. To aid in the interpretation of mortality data, the following observations were recorded: F (food left, assessed only on D8), S (small body size), A (abnormal moving behaviour in terms of increased activity), B (black spots or other discolorations indicating sickness), noted during scheduled assessments.

The Step-down Cochran-Armitage Test was used for statistical analysis of the adult emergence data and the estimation of the NOEC/NOED and LOEC/LOED as the data showed a monotonic trend. The accepted significance level was α = 0.05 (one-sided greater). The ED/EC10/20/50 values were determined with the Weibull analysis using linear maximum likelihood regression. The statistical calculations were performed with the statistical program ToxRat Professional 3.3.0 (Ratte, 2018).

Analytical method

Samples of feeding solution were analysed using the validated analytical method M-687032-01-1, report reference M-687032-01-1 (**see Doc MCP Section 5)**.

II. Results and Discussion

Validity criteria according to the OECD Guidance Document 239 (2016) were met:

- Larval mortality in the control: ≤ 15% for larvae across all control replicates (between day three and day eight) – (Actual: 5.6 %)
- Adult emergence rate: ≥ 70% for *Apis mellifera* L. across all control replicates (between day three and day 22) – (Actual: 83.3%)
- Larval mortality in the reference item treatment group: ≥ 50% for larvae across all reference replicates (between D3 and D8) – (Actual: 97.2%)

The concentrations of the active substances were determined in the diet samples. The mean recovery of prothioconazole and spiroxamine ranged between 83% and 109% in the final diets.

On day eight, a larval mortality of 5.6% was observed in the control (AC). In the test item group larval mortalities on day eight were 97.2%, 13.9%, 5.6%, 5.6% and 0.0% following a treatment with 300, 120, 48, 19.2 and 7.7 µg product/larva, respectively. Mortality of the reference item treated group (AR) was above 50% on day eight.

On day eight, none of the remaining larvae treated with the test item, were observed to have food left and/or a smaller body size.

In the final assessment on day 22, an adult emergence rate of 83.3% was determined for the honey bees in the control group. In the test item treated group the adult honey bees emerged at rates of 0.0%, 63.9%, 80.6%, 80.6% and 83.3% exposed to a cumulative dose 300, 120, 48, 19.2 and 7.7 µg product/larva, respectively, during the larval stages. On day 22, larvae treated with 300 and 120 µg product/larva, showed an emergence rate, which was statistically significantly different compared to the control.

The statistical evaluation of the adult emergence rate was done using absolute mortality data of the final assessment on day 22. The test item treatment groups were compared to the control (AC).

Table CP 10.3.1.3/01-1 Mortality and other observations of larvae and adults in the repeated exposure toxicity test on day 8

Treatment group	Treatment ID	Cumulative Dose (nominal)	Concentration (nominal)	On Day 8		
				Larval mortality D3 to D8 (%)		Mean OO (%)
		(µg product/ larva)	(mg product/ kg food)	abs.	corr.	
Control	AC	-	-	5.6	0.0	0.0
Test Item	AT	300	1896	97.2	97.1	0.0
	BT	120	758	13.9	8.8	0.0
	CT	48	303	5.6	0.0	0.0
	DT	19.2	121	5.6	0.0	0.0
	ET	7.7	49	0.0	0.0	0.0
Reference item		(µg a.s./ larva)	(mg a.s./kg food)			

Treatment group	Treatment ID	Cumulative Dose (nominal)	Concentration (nominal)	On Day 8		
				Larval mortality D3 to D8 (%)		Mean OO (%)
		(µg product/ larva)	(mg product/ kg food)	abs.	corr.	
	AR	7.6	48	97.2	97.1	0.0

Results are averages based on 3 replicates (hives), containing 12 larvae each; corr.: corrected mortality (according to Schneider-Orelli 1947): mortality in test and reference item treated groups were corrected by the mortality of the control (AC), respectively; abs.: absolute mortality as counted from the results; calculation were performed with non-rounded values; OO: Other observations (e.g. remaining food); negative values were set to "0"

* Statistically significant difference compared to control (Step-down Cochran-Armitage Test; p≤0.05; one sided greater)

Table CP 10.3.1.3/01-2 Mortality and other observations of larvae and adults in the repeated exposure toxicity test on day 22

Treatment group	Treatment ID	Cumulative Dose (nominal)	Concentration (nominal)	On day 22		
				Total mortality D3-D22 (%)		Adult emergence rate (%)
		(µg product/ larva)	(mg product/ Kg food)	abs.	corr.	abs.
Control	AC	-	-	16.7	0.0	83.3
Test Item	AT	300	1896	100.0	100.0	0.0*
	BT	120	758	30.1	23.3	63.9*
	CT	48	303	19.4	3.3	80.6
	DT	19.2	121	19.4	3.3	80.6
	ET	7.7	49	16.7	0.0	83.3
Reference item		(µg a.s./ larva)	(mg a.s./kg food)			
	AR	7.6	48	100.0	100.0	0.0

Results are averages based on 3 replicates (hives), containing 12 larvae each; corr.: corrected mortality (according to Schneider-Orelli 1947): mortality in test and reference item treated groups were corrected by the mortality of the control (AC), respectively; abs.: absolute mortality as counted from the results; calculation were performed with non-rounded values; OO: Other observations (e.g. remaining food); negative values were set to "0"

*Statistically significant difference compared to control (Step-down Cochran-Armitage Test; p≤0.05; one sided greater)

Table CP 10.3.1.3/01-3 Study endpoints

	Endpoints	Value
Test item cumulative doses	ED_{50} [µg product/larva]	151.6 (87.5 – 262.7)
	ED_{20} [µg product/larva]	99.0 (47.0 – 208.4)
	ED_{10} [µg product/larva]	74.6 (28.7 – 193.6)
	LOED [µg product/larva]	120
	NOED [µg product/larva]	48
Test item concentrations	EC_{50} [mg product/kg food]	958 (552 – 1661)
	EC_{20} [mg product/kg food]	625 (296 – 1318)
	EC_{10} [mg product/kg food]	471 (181 – 1225)
	LOEC [mg product/kg food]	758
	NOEC [mg product/kg food]	303

Values in parentheses are 95% confidence limits

III. Conclusion

In a 22-day repeated exposure larval toxicity study performed in a dose-response design with Prothioconazole + Spiroxamine EC 460 (160+300 g/L), the NOED and LOED was determined to be 48 µg and 120 µg product/larva (based on adult emergence), respectively. The NOEC and LOEC were 303 mg and 758 mg product/kg food, respectively.

The ED_{50}, ED_{20} and ED_{10} values (based on adult emergence) were determined to be 151.6 µg, 99.0 µg and 74.6 µg product/larva, respectively. The EC_{50}, EC_{20} and EC_{10} values were determined to be 958 mg, 625 mg and 471 mg product/kg food, respectively.

The mean recovery for prothioconazole and spiroxamine ranged between 83% and 109% in the final diets.

Assessment and conclusion by applicant:

Validity criteria according to the OECD Guidance Document 239 (2016), to which the study was conducted, were met:

- Larval mortality in the control: ≤ 15% for larvae across all control replicates (between day three and day eight) – (Actual: 5.6 %)
- Adult emergence rate: ≥ 70% for *Apis mellifera* L. across all control replicates (between day three and day 22) – (Actual: 83.3%)
- Larval mortality in the reference item treatment group: ≥ 50% for larvae across all reference replicates (between D3 and D8) – (Actual: 97.2%)

The study is therefore considered acceptable. Analytical measurements conformed the correct dosing of the larvae.

The NOED was determined to be 48 µg product/larva (based on adult emergence).

Data Point:	KCP 10.3.1.3/02
Report Author:	[redacted]; [redacted]
Report Year:	2020
Report Title:	Analytical phase report - Prothioconazole + spiroxamine EC 460 (160+300 g/L) - Repeated exposure to honey bee Larvae (Apis mellifera L.) under laboratory conditions
Report No:	19 48 BLC 0024-P1
Document No:	M-687032-01-1
Guideline(s) followed in study:	Regulation (EC) No 1107/2009 of the European Parliament and the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC European Commission Guidance Document for Generating and Reporting Methods of Analysis in Support of Pre-Registration Data Requirements for Annex II (Part A, Section 4) and Annex III (Part A, Section 5) of Directive 91/414, SANCO/3029/99 rev. 4, 11/07/00 Guidance document on residue analytical methods, SANCO/825/00 rev. 8.1, European Commission, Directorate General Health and Consumer Protection 16/11/2010 US EPA Residue Chemistry Guideline OCSPP 860.1340: Residue Analytical Method
Deviations from current test guideline:	This study was conducted by following an approved study plan. No deviation occurred in the analytical part of this study
Previous evaluation:	No, not previously submitted -
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The objective of this study was to determine the 22-day toxicity of the test item Prothioconazole + Spiroxamine EC 460 (160+300 g/L) to the honey bee, *Apis mellifera* L., larvae in an *in vitro* test after repeated exposure. The objective of the analytical phase of the study was to verify the concentration of prothioconazole and spiroxamine in the feeding diets.

The mean recovery values per fortification level of prothioconazole in feeding diets ranged between 89 and 110% with relative standard deviations between 5.0 and 11.2%. The overall mean recovery was 96% and the corresponding overall relative standard deviation (RSD) was 13.2% (n = 9).

The mean recovery values per fortification level of spiroxamine in feeding diets ranged between 88 and 107% with relative standard deviations between 0.6 and 10.6%. The overall mean recovery was 98% and the corresponding overall relative standard deviation (RSD) was 10.3% (n = 9).

I. Analytical method

Residues of prothioconazole and spiroxamine in/on bee relevant matrices were determined by HPLC-MS/MS according to method 01013-M001. The residues from bee relevant matrices were diluted with a mixture of acetonitrile/water. Afterwards, the stable isotopically labelled analytes were added. The solution was made up to volume, diluted where necessary and subjected to reversed phase HPLC-MS/MS. Prothioconazole was detected in the negative (ESI-) ion mode and spiroxamine was detected in the positive (ESI+) ion mode. Residues were quantified using internal stable labeled standards.

Sample preparation

A representative aliquot of 1 g of the sample was weighed into a 50 mL falcon tube, for recovery experiments fortify the weighed sample with the corresponding amount of test item and add 25 mL of acetonitrile/water (1/1; v/v) and 2 mL of a 250 g/L cysteine hydrochloride solution. The sample was

placed on the overhead shaker and the sample were shaked until the whole sample is solved. 0.05 mL of an internal standard solution containing 1000 µg/L was added and the solution was well shaken. The sample was transferred into a 50 mL volumetric flask and filled up to the final volume with acetonitrile/water (1/1; v/v). An aliquot of the sample solution was centrifuged at 13000 rpm for 5 minutes and subjected to HPLC-MS/MS determination. Samples containing high analyte concentrations were diluted until their concentrations were within the linearity range of the corresponding calibration curve.

For the feeding diets concurrent recovery determinations were included in each set of analyses (at least one recovery for ten study samples). The respective Limit of Quantification (LOQ) of prothioconazole and spiroxamine, defined as the lowest validated fortification level, was 0.01 mg/kg in feeding diets. The corresponding respective Limit of Detection (LOD) was 0.003 mg/kg.

HPLC-Instrument Conditions

An aliquot of the prepared sample was injected into the High-Performance Liquid Chromatograph (HPLC), chromatographed under gradient reversed phase conditions and detected by Tandem Mass Spectrometry (MS/MS) with electrospray ionization (ESI).

Mass Spectrometry

The detection by MS/MS was performed on a triple-quadrupole tandem mass spectrometer, equipped with a Turbo Ion Spray (ESI) interface operated in the positive and negative ion mode and multiple reaction monitoring mode (MRM). Unit mass resolution was established in the mass resolving quadrupoles by maintaining a full width at half-maximum (FWHM) of about 0.7 amu (= Unit Mass Resolution). Optimal collision-activated dissociation (CAD) conditions for fragmentation of the pseudomolecular ions of the analytes were applied with nitrogen as the collision gas.

Method Performance

Full validation data is documented within the method 01013/M001 itself for plant sample material. For the feeding diet a limited set of validation recoveries (one control sample, at least 3 repetitions each at two fortification levels) at the LOQ (0.01 mg/kg) and at the 10-fold LOQ level (0.10 mg/kg) was performed within this study. In order to check the performance of the method, concurrent recovery determinations were included in each set of analysis (at least one recovery for ten study samples). Additional concurrent recoveries were performed at 70000 x LOQ (3 x 700 mg/kg) for prothioconazole and 64000 x LOQ (3 x 640 mg/kg) for spiroxamine, respectively.

All results of the method validation were in accordance with the general requirements for residue analytical methods; therefore, the method was validated successfully.

Calculation of the residues

For calculation of the concentrations, calibration curves were used. These curves were calculated automatically after each sequence run with the Sciex quantitation software Analyst or LIMS.

The following equation was used for calculation of amounts using the internal standard procedure for a linear calibration curve of type y = ax + b:

$$C_S = \frac{\text{Area Ratio} - \text{Intercept (b)}}{\text{Slope (a)}} \cdot \frac{V_{End}}{M_S} \cdot DF$$

CS Concentration in the sample [mg/kg]

Area Ratio Peak area of the analyte in the sample solution [area counts], divided by the Peak area of the internal standard in the sample solution [area counts]

Intercept Point where the calibration line crosses the y-axis [area counts/area counts]

Slope Slope of the calibration line [(area counts/area counts) / (µg/L)]

=> (1/(µg/L) => L/µg (in this case IS Conc. is set to 1)

V_{End} Final volume of the extract [L]

M_s Mass of the extracted sample [g]

DF Optional Dilution Factor, depending on possible dilutions of the sample. Aliquots and final volumes [mL] have to be considered. If the sample was not diluted this value is 1

II. Analytical Results and Discussion

All residues in control samples from the diet solutions were below the LOD. Residues in the control and treated samples of feeding diets are shown in the following tables:

Table CP 10.3.1.3/02-1 Summary of prothioconazole in feeding diet

Sample-ID 1948BLC0024-	Actual conc. of protioconazole (mg/kg)	Target conc. of protioconazole (mg/kg)	Recovery from target** (%)
D3-AC-AE	< LOD	-	-
D3-AT-AE	295	308.5	96
D3-BT-AE	101	123.4	82
D3-CT-AE	40.9	49.4	83
D3-DT-AE	16.2	19.7	82
D3-ET-AE	6.81	7.90	74
D4-AC-AE	< LOD	-	-
D4-AT-AE	330	308.5	107
D4-BT-AE	115	123.4	93
D4-CT-AE	43.4	49.4	88
D4-DT-AE	19.3	19.7	98
D4-ET-AE	6.51	7.90	82
D5-AC-AE	< LOD	-	-
D5-AT-AE	310	308.5	100
D5-BT-AE	108	123.4	88
D5-CT-AE	44.6	49.4	90
D5-DT-AE	20.4	19.7	104
D5-ET-AE	7.02	7.90	89
D6-AC-AE	< LOD	-	-
D6-AT-AE	307	308.5	100
D6-BT-AE	108	123.4	88
D6-CT-AE	41.2	49.4	83

D6-DT-AE	17.9	19.7	91
D6-ET-AE	6.95	7.90	88

Table CP 10.3.1.3/02-2 Summary of spiroxamine in feeding diet

Sample-ID 1948BLC0024-	Actual conc. of protioconazole (mg/kg)	Target conc. of protioconazole (mg/kg)	Recovery from target* (%)
D3-AC-AE	< LOD	-	-
D3-AT-AE	572	578.5	99
D3-BT-AE	215	231.4	93
D3-CT-AE	92.5	92.6	100
D3-DT-AE	38.3	37.0	104
D3-ET-AE	14.9	14.8	101
D4-AC-AE	< LOD	-	-
D4-AT-AE	632	578.5	109
D4-BT-AE	229	231.4	99
D4-CT-AE	98.2	92.6	106
D4-DT-AE	40.2	37.0	109
D4-ET-AE	16.1	14.8	109
D5-AC-AE	< LOD	-	-
D5-AT-AE	588	578.5	109
D5-BT-AE	246	231.4	99
D5-CT-AE	97.7	92.6	106
D5-DT-AE	41.1	37.0	109
D5-ET-AE	16.0	14.8	109
D6-AC-AE	< LOD	-	-
D6-AT-AE	616	578.5	106
D6-BT-AE	234	231.4	101
D6-CT-AE	94.4	92.6	102
D6-DT-AE	41.2	37.0	111
D6-ET-AE	16.2	14.8	109

III. Evaluation and Discussion

The method validation was done with a set of recoveries at the LOQ (3 x 0.01 mg/kg, each) and 10 x LOQ (3 x 0.10 mg/kg, each) level. Additional concurrent recoveries were performed at 70000 x LOQ (3 x 700 mg/kg) for prothioconazole and 64000 x LOQ (3 x 640 mg/kg) for spiroxamine, respectively.

The mean recovery values per fortification level of prothioconazole in feeding diets ranged between 89 and 110% with relative standard deviations between 5.0 and 11.2%. The overall mean recovery was 96% and the corresponding overall relative standard deviation (RSD) was 13.2% (n = 9).

The mean recovery values per fortification level of spiroxamine in feeding diets ranged between 88 and 107% with relative standard deviations between 0.6 and 10.6%. The overall mean recovery was 98% and the corresponding overall relative standard deviation (RSD) was 10.3% (n = 9).

No residues of prothioconazole and spiroxamine above the LOD were found in any of the control bee larval diets.

Assessment and conclusion by applicant:

The study report presents the analytical method and the results of the analysis for the 22-day larval toxicity study with honeybees (M-756625-01-1). As such the study is considered to be acceptable.

Please refer to the Analytical Methods section of the dossier for a full assessment of the analytical method used.

CP 10.3.1.4 Sub-lethal effects

Additional studies on sub-lethal effects have not been conducted and are not considered to be necessary.

CP 10.3.1.5 Cage and tunnel tests

No data are available for Prothioconazole + Spiroxamine EC 460 but pollen and nectar decline trials have been conducted using Spiroxamine EC 500, under semi-field tunnel test conditions, which are also considered potentially relevant for the use of Prothioconazole + Spiroxamine EC 460 in cereals. A summary of the study has been provided below.

Data Point:	KCP 10.3.1.5/01
Report Author:	[redacted]
Report Year:	2021
Report Title:	Determination of residues of spiroxamine in nectar and pollen of Phacelia tanacetifolia after two applications of spiroxamine EC 500 in a semi-field tunnel residue study in Central and Southern Europe in 2020
Report No:	S20-02289
Document No:	M-763122-01-1
Guideline(s) followed in study:	Commission Regulation (EU) No 283/2013 and 284/2013 (Mar. 2013) in accordance with Regulation (EC) No 1107/2009 (Oct. 2009), SANCO/825/00 (2010), SANCO/3029/99 rev. 4 (2000), EC (2018) Technical guidelines for determining the magnitude of pesticide residues in honey and setting Maximum Residue Levels in honey (SANTE/11956/2016 rev. 9)
Deviations from current test guideline:	None
Previous evaluation:	No, not previously submitted -
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

Residues of spiroxamine were determined in nectar and pollen from *Phacelia tanacetifolia* plants under semi-field conditions. The study comprised five separate semi-field residue trials conducted in Germany (2 sites) and Spain (3 sites) in 2020. Each site comprised two plots (one untreated and one treated with Spiroxamine EC 500). The tunnels used per plot had an area of 200 m² each. Two bee hives were placed at the end of each tunnel. Spiroxamine EC 500 was applied at the nominal application rate of 0.6 L product/ha (corresponding to 300 g a.s./ha) in 200 L water/ha for both applications and all trials. On each sampling day forager bees were collected for the preparation of nectar from their honey stomachs for residue analysis. On each sampling day pollen from *Phacelia tanacetifolia* retrieved by the bees was collected using pollen traps. Sampling occurred shortly after application, 8 hours after application, 1, 2, 3, 5 and 7 days after application. Residues of spiroxamine enantiomers A1, A2, B1 and B2 were determined by HPLC-MS/MS detection. The Limit of Quantitation (LOQ), defined as the lowest validated fortification level, was 0.01 mg/kg (10 µg/kg, sum of four enantiomers) for all analysed samples. The corresponding respective Limit of Detection (LOD) was determined to be 0.003 mg/kg (3 µg/kg, sum of four enantiomers). Residues in control samples of pollen ranged between < 0.01 mg/kg and 0.0110 mg/kg (sum of four enantiomers). No residues of the analytes above the LOQ were found in any of the control samples of nectar. The residues found in pollen and nectar on the 5 tested sites are presented in the following tables.

Table CP 10.3.1.5/01-1 Residues of spiroxamine in pollen (sum of four enantiomers) found on each trial site [mg /kg]

Sample ID L20-02289	Sample type	Sample weight [g]	Residues of spiroxamine (sum of four enantiomers) [mg/kg]
Tria1S20-02289-01 (Germany)			
01-C-S1-P-A	C	0.220	< 0.01
01-C-S1-P-R	C	0.198	< 0.01

01-T-S1-P-A	T	0.200	87.0
01-T-S2-P-A	T	0.202	5.22
01-T-S3-P-A	T	0.201	3.40
01-T-S5-P-A	T	0.200	0.697
01-T-S6-P-A	T	0.250	2.10
01-T-S7-P-A	T	0.202	0.797
Trial S20-02289-02 (Germany)			
02-C-S1-P-A	C	0.202	< 0.01
02-C-S1-R-A	C	0.217	< 0.01
02-T-S1-P-A	T	0.201	71.9
02-T-S2-P-A	T	0.238	20.6
02-T-S3-P-A	T	0.202	3.63
02-T-S5-P-A	T	0.245	8.95
02-T-S6-P-A	T	0.201	0.193
02-T-S7-P-A	T	0.200	0.172
Trial S20-02289-03 (Spain)			
03-C-S1-P-A	C	0.211	0.0110
03-T-S1-P-A	T	0.201	37.9
03-T-S2-P-A	T	0.229	22.1
03-T-S3-P-A	T	0.201	4.59
03-T-S5-P-A	T	0.237	2.25
03-T-S6-P-A	T	0.201	1.13
03-T-S7-P-A	T	0.217	1.10
Trial S20-02289-04 (Spain)			
04-C-S1-P-A	C	0.201	< 0.01
04-T-S3-P-A	T	0.232	21.4
04-T-S5-P-A	T	0.200	1.35
04-T-S6-P-A	T	0.201	1.13

04-T-S7-P-A	T	0.200	0.280
Tria1S20-02289-05 (Spain)			
05-C-S1-P-A	C	0.197	< 0.01
05-T-S1-P-A	T	0.192	54.7
05-T-S2-P-A	T	0.130	14.1
05-T-S3-P-A	T	0.251	9.32
05-T-S5-P-A	T	0.200	1.23
05-T-S6-P-A	T	0.194	1.48
05-T-S7-P-A	T	0.191	0.569

C = Control, T = Treatment, LOQ = Limit of Quantification = 0.01 mg/kg (= 10 µg/kg = 10 ppb, sum of four enantiomers) for spiroxamine, LOD = Limit of Detection = 0.003 mg/kg (= 3 µg/kg = 3 ppb, sum of four enantiomers) for spiroxamine

Table CP 10.3.1.5/01-2 Residues of spiroxamine in nectar (sum of four enantiomers) found on each trial site [mg /kg]

Sample ID L20-02289	Sample type	Sample weight [g]	Residues of Spiroxamine (sum of four enantiomers) [mg/kg]
Tria1S20-02289-01 (Germany)			
01-C-S1-NFB-A	C	0.200	< 0.01
01-T-S1-NFB-A	T	0.200	0.700
01-T-S2-NFB-A	T	0.200	0.252
01-T-S3-NFB-A	T	0.200	0.116
01-T-S4-NFB-A	T	0.200	0.0268
01-T-S5-NFB-A	T	0.200	0.00943
01-T-S6-NFB-A	T	0.186	< 0.01
01-T-S7-NFB-A	T	0.200	< 0.01
Tria1S20-02289-02 (Germany)			
02-C-S1-NFB-A	C	0.200	< 0.01
02-T-S1-NFB-A	T	0.200	0.163
02-T-S2-NFB-A	T	0.201	0.195

02-T-S3-NFB-A	T	0.200	0.0313
02-T-S4-NFB-A	T	0.200	< 0.01
02-T-S5-NFB-A	T	0.200	< 0.01
02-T-S6-NFB-A	T	0.200	< 0.01
02-T-S7-NFB-A	T	0.200	< 0.01
Trial S20-02289-03 (Spain)			
03-C-S1-NFB-A	C	0.200	< 0.01
03-T-S1-NFB-A	T	0.200	0.117
03-T-S2-NFB-A	T	0.200	0.0757
03-T-S3-NFB-A	T	0.200	0.0561
03-T-S4-NFB-A	T	0.199	0.0247
03-T-S5-NFB-A	T	0.200	< 0.01
03-T-S6-NFB-A	T	0.200	< 0.01
03-T-S7-NFB-A	T	0.200	< 0.01
Trial S20-02289-04 (Spain)			
04-C-S1-NFB-A	C	0.183	< 0.01
04-T-S1-NFB-A	T	0.200	0.221
04-T-S2-NFB-A	T	0.200	0.445
04-T-S3-NFB-A	T	0.200	0.104
04-T-S4-NFB-A	T	0.200	0.0193
04-T-S5-NFB-A	T	0.200	< 0.01
04-T-S6-NFB-A	T	0.200	< 0.01
04-T-S7-NFB-A	T	0.200	< 0.01
Trial S20-02289-05 (Spain)			
05-C-S1-NFB-A	C	0.200	< 0.01
05-T-S1-NFB-A	T	0.200	0.0471
05-T-S2-NFB-A	T	0.133	0.0777
05-T-S3-NFB-A	T	0.163	0.0704

05-T-S4-NFB-A	T	0.200	0.0128
05-T-S5-NFB-A	T	0.200	< 0.01
05-T-S6-NFB-A	T	0.200	< 0.01
05-T-S7-NFB-A	T	0.168	< 0.01

C = Control, T = Treatment, LOQ = Limit of Quantification = 0.01 mg/kg (= 10 µg/kg = 10 ppb, sum of four enantiomers) for spiroxamine, LOD = Limit of Detection = 0.003 mg/kg (= 3 µg/kg = 3 ppb, sum of four enantiomers) for spiroxamine

I. Materials and Methods

Materials

Study code:	S20-02289-01	S20-02289-02	S20-02289-03	S20-02289-04	S20-02289-05
Test Material	Spiroxamine EC 500				
Lot/Batch #:	EM4L027093				
Actual content of active ingredients:	49.8 % w/w, 500 g/L (nominal) 49.2 % w/w, 494.1 g/L (analysed)				
Description:	Dark yellow, clear liquid				
Stability of test compound:	Sufficient for the test purpose				
Reanalysis/Expiry date:	January 22, 2024				
Density:	1.004 g/cm³ (analysed)				

Treatments

Test rates:	Nominal: 0.6 L product/ha (corresponding to 300 g a.s./ha) in 200 L water/ha				
Vehicle:	Tap water				
Application:	Calibrated boom sprayer (2.5 m) - 5 flat fan, 50 cm spacing (XR 110 01 VS)		Calibrated bar sprayer sprayer - 5 / HYPRO green (F110-015)		

Test design

Test system:	*Phacelia tanacetifolia*	*Phacelia tanacetifolia*	*Phacelia tanacetifolia*	*Phacelia tanacetifolia*	*Phacelia tanacetifolia*
Cultivar / Variety:	Balo	Natra	Stala	Stala	Stala

Location:	76297, Stutensee, Baden-Württemberg, Germany	75177, Pforzheim, Baden-Württemberg, Germany	46220, Picessent, Valencia, Spain	46820, Anna, Valencia, Spain	02640 Almansa, Albacete, Spain
Distance between trials:	> 20 km	> 20 km	> 20 km	> 20 km	> 20 km
Planting or seeding date:	2020-03-19	2020-06-08	2020-02-11	2020-02-05	2020-05-26
Seeds per ha:	15 kg seeds/ha	15 kg seeds/ha	10 kg seeds/ha	10 kg seeds/ha	10 kg seeds/ha
Plot size (width x length):	5 m x 40 m	5 m x 40 m	5 m x 40 m	5 m x 40 m	5 m x 40 m
Treated area:	185 m²	185 m²	169.4 m²	169.4 m²	169.4 m²
Closest distance between control and treated plot(s):	10 m	10 m	20 m	25.7 m	27.3 m
Minimum distance to the edge of the field:	>3 m	>3 m	6.7 m	2 m	12.45 m
Soil type (USDA):	Loamy sand	Silt	Sandy loam	Sandy clay loam	Sandy loam
Test organisms:	Honeybee colonies (*Apis mellifera* L.)				
Environmental test conditions					
Temperature (°C):	2.0 - 29.2	7.9 - 36.9	9.7 - 32.3	8.7 - 35.9	11.8 - 40.8
Humidity (%):	26.2 - 93.7	20.1 - 99.8	0.0 - 100.0	23.0 - 100.0	15.6 - 100.0
Daily precipitation (mm):	0.0 - 7.1	0.0 - 24.0	0.0 - 4.0	0.0 - 14.06	0.0 - 0.8

Study Design

Residues of spiroxamine were determined in nectar and pollen from *Phacelia tanacetifolia* plants under semi-field conditions. The study comprised five separate semi-field residue trials conducted in Germany (2 sites) and Spain (3 sites) in 2020 (please refer to the table above for details on the location and field sites). Each site comprised two plots (one untreated and one treated with Spiroxamine EC 500). Specifications of the plots were provided in the above table per site. The tunnels used per plot had an area of 200 m² each, with 2 rows of treated *P. tanacetifolia* (2.2 m x 37.0 m) divided by a 0.6 m uncultivated inter-row. Two bee hives and a water supply were placed at the end and middle of each tunnel respectively. Weather data (air temperature, humidity and precipitation) were recorded at the field site of each trial. During sowing and residue sampling the climatic conditions were measured with

portable equipment or weather stations at the trial sites (GLP data). For the period between sowing and start of measurement at the field site weather data from an official weather station were taken (non-GLP).

Spiroxamine EC 500 was applied using a calibrated sprayer. The nominal application rate was 0.6 L product/ha (corresponding to 300 g a.s./ha) in 200 L water/ha for both applications and all trials. Before application, the sprayer was calibrated and the duration of spraying per plot was calculated according to the output. The actual amounts of the product and spray volume applied were determined by recording the amount of spray solution prepared and the amount remaining after the application. The application rate of the active substance was calculated based on the nominal content and density. No additional adjuvants, surfactants or mixing partners were used for the application. Actual applied spray volume was within a spray tolerance of ±10 %. For all trials and both applications the deviations ranged between -2.23 % and +3.66 %.

Sample processing:

On each sampling day forager bees were collected for the preparation of nectar from their honey stomachs for residue analysis. The hive entrances were sealed before the sampling and the forager bees were subsequently collected as they returned to the hive using modified louvers ("bee vac"), or using tweezers if only few bees are returning. After sampling, the hives were re-opened. On each sampling day an A-sample of at least 150 bees was collected. If possible an R-sample of at least 150 bees was taken on each sampling day, too.

For the preparation of nectar from honey stomachs for determination of sugar content, forager bees were sampled in the control on each sampling day. One sample of at least 50 bees was taken per sampling day. No R-sample was taken.

On each sampling day pollen from *Phacelia tanacetifolia* retrieved by the bees was collected using pollen traps. The hives in each tunnel were equipped with pollen traps. Bees strip off the pollen when passing a grid. This pollen grid was only inserted on sampling days. After collection of the pollen the grid was removed. On each sampling day an A-sample and an R-sample of at least 0.2 g pollen was collected.

Control samples were taken before the test item treatment samples or were taken by different personnel, and different equipment was used.

All samples were transported on dry ice from the field to the test facility/test site. Samples were stored deep frozen within 12 hours after sampling. The field samples were stored in a freezer at -18 °C or below until preparation of the examination samples. The forager bees were shipped to the Study Director for preparation of honey stomachs and sugar content determination. The maximum storage interval from sampling to extraction was 164 days. Storage at the Analytical Test Site from sample receipt until lab sample preparation was at ≤ -18 °C. The maximum interval from extraction to analysis at 1 °C to 10 °C with given exceptions was 2 days.

For the preparation of honey stomachs from forager bees for residue analysis the total amount of bees per sample was counted. At least 75 bees of the A-sample were prepared. If the minimum amount of prepared nectar was not obtained from the sub-sample A, sub-sample R was prepared and added to sub-sample A, until the requested amount of 200 mg nectar was achieved. The duration of any samples remaining outside of the freezer did not exceed 2 hours. Honeybees from the control group (C) were processed first. Once this task was completed, then the process was started with the honeybees from the test item treatment group (T). The total number of prepared honeybees and the sub-samples used was recorded. For the preparation of honey stomachs from forager bees for sugar content determination the total amount of honeybees per sample was counted. The amount of at least 12 forager bees was prepared. The sugar content was determined immediately after preparation by a digital refractometer in the laboratory.

Sample schedule

Sampling of the different matrices was performed according to the following schedule:

Table CP 10.3.1.5/01-3 Matrices sampling schedule for trials S20-02289-01 to -05:

Sampling code	Timing	Treatment/ Plot	Commodity	Quantity (min) per subsample		Sample type
				A	R	
S1	0DAA2 (shortly after application)	C, T	Forager bees	150	150	Residue
			Forager bees	50	-	Sugar content
			Pollen	0.2 g	0.2 g	Residue
S2	0DAA2 (8 h after application)	T	Forager bees	150	150	Residue
			Forager bees	50	-	Sugar content
			Pollen	0.2 g	0.2 g	Residue
S3	1DAA2	T	Forager bees	150	150	Residue
			Forager bees	50	-	Sugar content
			Pollen	0.2 g	0.2 g	Residue
S4	2DAA2	T	Forager bees	150	150	Residue
			Forager bees	50	-	Sugar content
S5	3(±1)DAA2	T	Forager bees	150	150	Residue
			Forager bees	50	-	Sugar content
			Pollen	0.2 g	0.2 g	Residue
S6	5(±1)DAA2	T	Forager bees	150	150	Residue
			Forager bees	50	-	Sugar content
			Pollen	0.2 g	0.2 g	Residue
S7	7(±1)DAA2	T	Forager bees	150	150	Residue
			Forager bees	50	-	Sugar content
			Pollen	0.2 g	0.2 g	Residue

DAA: days after application

Residue analysis and analytic methods

The analytical method M01480/M001 was developed to determine the residues of spiroxamine (AE 1344293) in/on honey, pollen and nectar as sum of its four enantiomers A1, A2, B1 and B2 by HPLC–MS/MS detection.

The samples were diluted/extracted with a methanol/water mixture (3/1, v/v). After filtration of the raw extract, an aliquot was analyzed by high performance liquid chromatography, chromatographed under chiral reverse phase column chromatography and detected by Tandem Mass Spectrometry with electrospray ionisation. Residues were quantified using solvent standards with an isotopic stable-labelled internal standard. For details on sample preparation for pollen and nectar please refer to the study report.

The method validation was done with a set of recoveries at the LOQ (5 x 0.01 mg/kg, sum of four enantiomers) and 10 x LOQ (5 x 0.10 mg/kg, sum of four enantiomers) level.

Full validation data is documented within the method 01480/M001 (chiral method) for pollen and nectar. A full set of validation recoveries (one control sample, at least 5 repetitions each at two fortification levels) at the LOQ (0.01 mg/kg, sum of four enantiomers) and at the 10-fold LOQ level (0.10 mg/kg, sum of four enantiomers) was also performed within this study, corresponding to 0.0027 mg/kg and 0.027 mg/kg for enantiomers A1 and A2 and 0.0023 mg/kg and 0.023 mg/kg for enantiomers B1 and B2 (chiral method). In order to check the performance of the methods, concurrent recovery determinations were included in each set of analyses (at least one recovery for ten study samples).

Recoveries were performed by spiking pollen and nectar with the test items. For control material pollen provided by the laboratory and synthetic nectar (prepared by dissolving 24.0 g glucose and 12.0 g fructose in water and filling up to 100 mL with water) was used for validation and concurrent recoveries.

The apparent residues in the control samples used for the performance of recoveries were below 30% of the LOQ. Recoveries were not corrected for apparent residues in the control samples used for these recoveries.

The mean recovery values (method validation) of the spiroxamine enantiomers (chiral method) in pollen ranged between 98% and 107% with relative standard deviations between 3.3% and 12.3%. The overall mean recoveries of the analytes ranged between 98% and 104% and the corresponding overall relative standard deviation (RSD) ranged between 10.9% and 12.6% (n = 10 for each analyte).

The mean recovery values (method validation) of the spiroxamine enantiomers (chiral method) in nectar ranged between 91% and 105% with relative standard deviations between 2.6% and 12.5%. The overall mean recoveries of the analytes ranged between 94% and 100% and the corresponding overall relative standard deviation (RSD) ranged between 6.4% and 11.2% (n = 10 for each analyte).

The Limit of Quantitation (LOQ), defined as the lowest validated fortification level, was 0.01 mg/kg (10 µg/kg, sum of four enantiomers) for all analysed samples. The corresponding respective Limit of Detection (LOD) was determined to be 0.003 mg/kg (3 µg/kg, sum of four enantiomers). Therefore, all results of the concurrent recoveries were in accordance with the general requirements for residue analytical methods.

Analytical method

Samples of nectar and pollen were analysed using the validated analytical method 01480/M001, report reference M-763118-01-1 **(see Doc MCA Section 4)**.

II. Results and Discussion

Residues in control samples of pollen ranged between <0.01 mg/kg and 0.0110 mg/kg (sum of four enantiomers). No residues of the analytes above the LOQ were found in any of the control samples of nectar. The residues found in the control and treated nectar and pollen samples are shown in the following tables. The results were not corrected for concurrent recoveries.

Table CP 10.3.1.5/01-4 Summary of residues of spiroxamine in pollen (sum of four enantiomers) found on each trial site [mg/kg]

Sample ID L20-02289	Sample type	Sample weight [g]	Residues of Spiroxamine (sum of four enantiomers) [mg/kg]
Tria1S20-02289-01 (Germany)			
01-C-S1-P-A	C	0.220	< 0.01
01-C-S1-P-R	C	0.198	< 0.01

Sample ID L20-02289	Sample type	Sample weight [g]	Residues of Spiroxamine (sum of four enantiomers) [mg/kg]
01-T-S1-P-A	T	0.200	87.0
01-T-S2-P-A	T	0.202	5.22
01-T-S3-P-A	T	0.201	3.40
01-T-S5-P-A	T	0.200	0.697
01-T-S6-P-A	T	0.250	2.10
01-T-S7-P-A	T	0.202	0.797
Trial S20-02289-02 (Germany)			
02-C-S1-P-A	C	0.202	< 0.01
02-C-S1-R-A	C	0.217	< 0.01
02-T-S1-P-A	T	0.201	71.9
02-T-S2-P-A	T	0.238	20.6
02-T-S3-P-A	T	0.202	3.63
02-T-S5-P-A	T	0.245	8.95
02-T-S6-P-A	T	0.201	0.193
02-T-S7-P-A	T	0.200	0.172
Trial S20-02289-03 (Spain)			
03-C-S1-P-A	C	0.211	0.0110
03-T-S1-P-A	T	0.201	37.9
03-T-S2-P-A	T	0.229	22.1
03-T-S3-P-A	T	0.201	4.59
03-T-S5-P-A	T	0.237	2.25
03-T-S6-P-A	T	0.201	1.13
03-T-S7-P-A	T	0.217	1.10
Trial S20-02289-04 (Spain)			
04-C-S1-P-A	C	0.201	< 0.01
04-T-S3-P-A	T	0.232	21.4

Sample ID L20-02289	Sample type	Sample weight [g]	Residues of Spiroxamine (sum of four enantiomers) [mg/kg]
04-T-S5-P-A	T	0.200	1.35
04-T-S6-P-A	T	0.201	1.13
04-T-S7-P-A	T	0.200	0.280
Trial S20-02289-05 (Spain)			
05-C-S1-P-A	C	0.197	< 0.01
05-T-S1-P-A	T	0.192	54.7
05-T-S2-P-A	T	0.130	14.1
05-T-S3-P-A	T	0.251	9.32
05-T-S5-P-A	T	0.200	1.23
05-T-S6-P-A	T	0.194	1.48
05-T-S7-P-A	T	0.191	0.569

C = Control, T = Treatment, LOQ = Limit of Quantification = 0.01 mg/kg (= 10 µg/kg = 10 ppb, sum of four enantiomers) for spiroxamine, LOD = Limit of Detection = 0.003 mg/kg (= 3 µg/kg = 3 ppb, sum of four enantiomers) for spiroxamine

Table CP 10.3.1.5/01-5 Summary of residues of spiroxamine in nectar (sum of four enantiomers) found on each trial site [mg/kg]

Sample ID L20-02289	Sample type	Sample weight [g]	Residues of Spiroxamine (sum of four enantiomers) [mg/kg]
Trial S20-02289-01 (Germany)			
01-C-S1-NFB-A	C	0.200	< 0.01
01-T-S1-NFB-A	T	0.200	0.700
01-T-S2-NFB-A	T	0.200	0.252
01-T-S3-NFB-A	T	0.200	0.116
01-T-S4-NFB-A	T	0.200	0.0268
01-T-S5-NFB-A	T	0.200	0.00943
01-T-S6-NFB-A	T	0.186	< 0.01
01-T-S7-NFB-A	T	0.200	< 0.01

Sample ID L20-02289	Sample type	Sample weight [g]	Residues of Spiroxamine (sum of four enantiomers) [mg/kg]
Trial S20-02289-02 (Germany)			
02-C-S1-NFB-A	C	0.200	< 0.01
02-T-S1-NFB-A	T	0.200	0.163
02-T-S2-NFB-A	T	0.201	0.195
02-T-S3-NFB-A	T	0.200	0.0313
02-T-S4-NFB-A	T	0.200	< 0.01
02-T-S5-NFB-A	T	0.200	< 0.01
02-T-S6-NFB-A	T	0.200	< 0.01
02-T-S7-NFB-A	T	0.200	< 0.01
Trial S20-02289-03 (Spain)			
03-C-S1-NFB-A	C	0.200	< 0.01
03-T-S1-NFB-A	T	0.200	0.117
03-T-S2-NFB-A	T	0.200	0.0757
03-T-S3-NFB-A	T	0.200	0.0562
03-T-S4-NFB-A	T	0.199	0.0247
03-T-S5-NFB-A	T	0.200	< 0.01
03-T-S6-NFB-A	T	0.200	< 0.01
03-T-S7-NFB-A	T	0.200	< 0.01
Trial S20-02289-04 (Spain)			
04-C-S1-NFB-A	C	0.183	< 0.01
04-T-S1-NFB-A	T	0.200	0.221
04-T-S2-NFB-A	T	0.200	0.445
04-T-S3-NFB-A	T	0.200	0.104
04-T-S4-NFB-A	T	0.200	0.0193
04-T-S5-NFB-A	T	0.200	< 0.01
04-T-S6-NFB-A	T	0.200	< 0.01

Sample ID L20-02289	Sample type	Sample weight [g]	Residues of Spiroxamine (sum of four enantiomers) [mg/kg]
04-T-S7-NFB-A	T	0.200	< 0.01
Trial S20-02289-05 (Spain)			
05-C-S1-NFB-A	C	0.200	< 0.01
05-T-S1-NFB-A	T	0.200	0.0471
05-T-S2-NFB-A	T	0.133	0.0777
05-T-S3-NFB-A	T	0.163	0.0704
05-T-S4-NFB-A	T	0.200	0.0128
05-T-S5-NFB-A	T	0.200	< 0.01
05-T-S6-NFB-A	T	0.200	< 0.01
05-T-S7-NFB-A	T	0.168	< 0.01

C = Control, T = Treatment, LOQ = Limit of Quantification = 0.01 mg/kg (= 10 µg/kg = 10 ppb, sum of four enantiomers) for spiroxamine, LOD = Limit of Detection = 0.003 mg/kg (= 3 µg/kg = 3 ppb, sum of four enantiomers) for spiroxamine

Sugar content determination

The sugar content of nectar sampled from forager bees was determined by a digital refractometer. The sugar content was in a range from 30.1% to 44.4% for Trial 01, from 21.1% to 61.0% for Trial 02, from 11.9% to 42.2% for Trial 03, from 12.3% to 44.1% for Trial 04 and from 8.7% to 32.3% for Trial 05.

III. Conclusion

The mean recovery values (method validation) of the spiroxamine enantiomers (chiral method) in pollen ranged between 98% and 107% with relative standard deviations between 3.3% and 17.3%. The overall mean recoveries of the analytes ranged between 98% and 104% and the corresponding overall relative standard deviation (RSD) ranged between 10.9% and 12.6% (n = 10 for each analyte).

The mean recovery values (method validation) of the spiroxamine enantiomers (chiral method) in nectar ranged between 91% and 105% with relative standard deviations between 2.6% and 12.5%. The overall mean recoveries of the analytes ranged between 94% and 100% and the corresponding overall relative standard deviation (RSD) ranged between 6.4% and 11.2% (n = 10 for each analyte).

The Limit of Quantitation (LOQ), defined as the lowest validated fortification level, was 0.01 mg/kg (10 µg/kg, sum of four enantiomers) for all analysed samples. The corresponding respective Limit of Detection (LOD) was determined to be 0.003 mg/kg (3 µg/kg, sum of four enantiomers).

Assessment and conclusion by applicant:

The study followed the analytical Guidance Document, SANCO/3029/99 rev. 4 and the criteria for method validation were all met. Thus, the analytical results are considered to be valid and acceptable for use in the risk assessment.

The study was conducted taking in to consideration the requirments of modern guidance on residues decline trials. The sampling regime was considered to be suitable (0 hours, 8 hours, 1, 2, 3, 5 and 7 days after application) as frequent sampling timepoints were used which spanned the estimated DT_{50} value therefore the results are considered suitable for kinetic modelling for DT_{50} values.

Five trials were conducted therefore it is considered that a sufficient number of trials are available in order to derive mean DT_{50} values for spiroxamine in pollen and in nectar for use in a risk assessment.

The study was conducted in *Phacelia* which was chosen as it is known to be a bee attractive crop and one that produces both nectar and pollen.

The study is considered to be acceptable.

CP 10.3.1.6 Field tests with honeybees

No data are available for Prothioconazole + Spiroxamine EC 460.

CP 10.3.2 Effects on non-target arthropods other than bees

The table below summarises the available data for non-target arthropods, all of which has been conducted using the representative formulation Prothioconazole + Spiroxamine EC 460. Extended test and aged residues data are available with a range of foliar and one soil NTA species.

Table CP 10.3.2-1 Summary of NTA studies with Prothioconazole + Spiroxamine EC 460

Organism	Test item	Test type	Endpoints		Reference
Typhlodromus pyri	Prothioconazole + Spiroxamine EC 460	Tier II Extended laboratory test; natural substrate (2D) – bean leaves	LR_{50} >2.5 L product/ha ER_{50} >2.5 L product/ha	EU	M-059177-01-1
Typhlodromus pyri	Prothioconazole + Spiroxamine EC 460	Tier II Extended laboratory test; aged residues; natural substrate (3D) – maize plants	LR_{50} and ER_{50} >2 x 1.25 L product/ha for Day 0 and Day 7 bioassays	EU	M-059404-01-1
Aphidius rhopalosiphi	Prothioconazole + Spiroxamine EC 460	Tier II Extended laboratory test; natural substrate (3D) – maize plants	LR_{50} >1.25 L product/ha ER_{50} >1.25 L product/ha	EU	M-065998-01-1
Aphidius rhopalosiphi	Prothioconazole + Spiroxamine EC 460	Tier II Extended laboratory test; aged residues; natural substrate (3D) – barley seedlings	LR_{50} and ER_{50} >3 x 1.25 L product/ha for Day 0 and Day 7 bioassays	EU	M-259098-01-1
Aphidius rhopalosiphi	Prothioconazole + Spiroxamine EC 460	Tier II Extended laboratory test; aged residues; natural substrate (3D) – maize plants	LR_{50} and ER_{50} >2 x 1.25 L product/ha for Day 0 and Day 7 bioassays	EU	M-056762-01-1
Coccinella septempunctata	Prothioconazole + Spiroxamine EC 460	Tier II Extended laboratory test; natural substrate (2D) – bean leaves	LR_{50} >2.875 L product/ha ER_{50} >2.875 L product/ha	EU	M-259116-01-1

Organism	Test item	Test type	Endpoints		Reference
Aleochara bilineata	Prothioconazole + Spiroxamine EC 460	Tier II Extended laboratory test; (2D)	LR_{50} >2.5 L product/ha ER_{50} >2.5 L product/ha	EU	M-298137-01-1

EU: previously evaluated as part of the original EU review and listed in EFSA conclusion and DAR

Prothioconazole endpoints

The EFSA Conclusion for prothioconazole (EFSA Scientific Report (2007) 106, 1-98) provides NTA endpoints which have been conducted with a 250 EC formulation of prothioconazole which are not considered to be relevant for the risk assessment of Prothioconazole + Spiroxamine EC 460. The data generated using Prothioconazole + Spiroxamine EC 460 are considered to be the most relevant and have therefore been used in the risk assessment.

Isomers

In terms of organism exposure, the critical highest predicted concentrations in the environment will occur immediately or very shortly after application therefore the effects of potential changes in the isomer ratios over time in the environment are not considered to be relevant to the NTA risk assessment. However, even if exposure to residues over a prolonged period of time were to occur, according to the current residues data set for spiroxamine there are no indications of a significant change in isomer ratios therefore no additional factor need be applied to the risk assessments below (*i.e.* an UF of 1.0 has been used).

Risk assessment

The evaluation of the risk for non-target arthropods was performed in accordance with the recommendations of the "Guidance Document on Terrestrial Ecotoxicology", as provided by the Commission Services (SANCO/10329/2002 rev.2 (final), October 17, 2002), and in consideration of the recommendations of the guidance document ESCORT 2. As required, a risk assessment for both in-field and off-field exposure have been conducted.

In-field exposure

The representative GAP for Prothioconazole + Spiroxamine EC 460 includes either a single or two applications of 1.25 L product/ha to cereals therefore both of these uses have been considered in the risk assessment.

The in-field exposure (predicted environmental rate, PER) is calculated according to ESCORT 2 using the following equation:

$$PER_{IN\text{-}FIELD} = \text{Application rate (L product/ha)} \times MAF$$

The MAF is a generic multiple application factor which is used to take in to account the potential build-up of applied substances between applications based on the application interval, DT_{50} value and number of applications.

The maximum in-field exposure (Predicted Environmental Rate, $PER_{IN\text{-}FIELD}$) to foliar-dwelling or soil-dwelling organisms assumes the worst-case scenarios of 100% crop interception and 0% crop interception, respectively.

The predicted exposure rate (PER) for in-field exposure of both foliar and soil-dwelling non-target arthropods for the uses in cereals (1 x 1.25 L product/ha and 2 x 1.25 L product/ha) has been calculated according to ESCORT II and summarised in the table below.

Table CP 10.3.2-2 PER for in-field exposure following the uses of Prothioconazole + Spiroxamine EC 460

Crop	Application rate (L product/ha)	Foliar		Soil	
		MAF[1]	$PER_{IN-FIELD}$ (L product/ha)	MAF[1]	$PER_{IN-FIELD}$ (L product/ha)
Cereals	1 x 1.25	1.0	1.25	1.0	1.25
	2 x 1.25	1.7	2.13	1.9	2.38

[1]: MAF = Multiple Application Factor (Appendix III of ESCORT II)

Tier I standard laboratory glass plate data using Prothioconazole + Spiroxamine EC 460 are not available therefore no Tier I risk assessment has been presented. Instead, a Tier II risk assessment has been conducted. This is considered to be fully acceptable because extended laboratory test data are available with the two indicator species, *T. pyri* and *A. rhopalosiphi*, as well as extended test data with two additional species, *C. septempunctata* and *A. bilineata*. Thus, even if a Tier I risk assessment were not to pass, Tier II extended data with the required number of species are available.

For the extended laboratory Tier II studies the risk is considered acceptable if the $PER_{IN-FIELD}$ concentrations are below the test concentrations resulting in 50% effects.

The Tier II in-field risk assessments for one and two applications of 1.25 L product/ha to cereals are presented in Table 10.3.2-3 and Table 10.3.2-4, respectively.

Table CP 10.3.2-3 Tier II in-field risk assessment for the proposed use of Prothioconazole + Spiroxamine EC 460 in cereals (1 x 1.25 L product/ha)

Intended use	Cereals		
Product	Prothioconazole + Spiroxamine EC 460		
Application rate (L product/ha)	1 × 1.25		
MAF	1.0		
Species	**LR_{50}/ER_{50} (L product/ha)**	**$PER_{in-field}$ (L product/ha)**	**<50% effects at predicted rate?**
Aphidius rhopalosiphi	>1.25	1.25	Y
Aphidius rhopalosiphi	>1.25 (2 x apps)		Y
Aphidius rhopalosiphi	>1.25 (3 x apps)		Y
Typhlodromus pyri	>2.5		Y
Typhlodromus pyri	>1.25 (2 x apps)		Y
Coccinella septempunctata	>2.875		Y
Aleochara bilineata	>2.5		Y

MAF: Multiple application factor; PER: Predicted environmental rate

Table CP 10.3.2-4 Tier II in-field risk assessment for the proposed use of Prothioconazole + Spiroxamine EC 460 in cereals (2 x 1.25 L product/ha)

Intended use	Cereals		
Product	Prothioconazole + Spiroxamine EC 460		
Application rate (L product/ha)	2 × 1.25		
MAF	1.7 (foliar); 1.9 (soil)		
Species	**LR_{50}/ER_{50} (L product/ha)**	**$PER_{in\text{-}field}$ (L product/ha)**	**<50% effects at predicted rate?**
Aphidius rhopalosiphi	>1.25	Foliar: 2.13 Soil: 2.38	N [1]
Aphidius rhopalosiphi	>1.25 (2 x apps)		Y [2]
Aphidius rhopalosiphi	>1.25 (3 x apps)		Y [2]
Typhlodromus pyri	>2.5		Y
Typhlodromus pyri	>1.25 (2 x apps)		Y [2]
Coccinella septempunctata	>2.875		Y
Aleochara bilineata	>2.5		Y

MAF: Multiple application factor; PER: Predicted environmental rate

[1] Acceptable risks cannot be confirmed however there are two additional studies with *Aphidius* which confirm that there are <50% effects following two applications of 1.25 L product/ha

[2] Study design incorporated multiple applications of 1.25 L product/ha therefore the results confirm there to be <50% effects following two or three applications of 1.25 L product/ha

For the single application to cereals it is clear that there are less than 50% effects at the $PER_{in\text{-}field}$ because all LR_{50} and ER_{50} values are >1.25 L product/ha. The in-field risk to NTA populations is therefore acceptable following a single application of Prothioconazole + Spiroxamine EC 460 to cereals.

For two applications to cereals it is not possible to make an assessment based on two of the available studies as the LR_{50} and ER_{50} values were >1.25 L product/ha which is not higher than the $PER_{in\text{-}field}$ values. However, for these species there are additional data in which multiple applications were tested in the study itself therefore an assessment can be made based upon these results. It is considered that less than 50% effects, following two applications of 1.25 L product/ha, have been sufficiently demonstrated for all of the four tested species. Thus, the in-field risks to NTA populations are therefore acceptable following two applications of Prothioconazole + Spiroxamine EC 460 to cereals.

Off-field exposure

Effects on non-target terrestrial plants are of concern in the off-field environment, where plants may be exposed to spray drift. The amount of spray drift reaching off-crop habitats is calculated using the appropriate percentile estimates, which depends on the number of applications, and is derived from the BBA (2000[13]) values from the spray drift predictions of Ganzelmeier & Rautmann (2000[14]).

Off-field foliar PER values have been calculated from in-field foliar PERs in conjunction with drift values as shown in the following equation:

[13] BBA (2000) Bundesanzeiger Jg. 52 (Official Gazette), Nr 100, S. 9879-9880 (25.05.2000) Bekanntmachung über die Abtrifteckwerte, die bei der Prüfung und Zulassung von Pflanzenschutzmitteln herangezogen werden. Public domain.

[14] Ganzelmeier H., Rautmann D. (2000) Drift, drift-reducing sprayers and sprayer testing. Aspects of Applied Biology 57, 2000, Pesticide Application. Public domain.

$$\text{Off-field PER} = \frac{\text{PER}_{\text{IN-FIELD}} \text{ x (\% drift/100)}}{\text{Vegetation distribution factor}}$$

The model used to estimate spray drift was developed for drift onto a two-dimensional water surface and, as such, does not account for interception and dilution by three-dimensional vegetation in off-crop areas. Therefore, a vegetation distribution or dilution factor is incorporated into the equation when calculating PERs to be used in conjunction with toxicity endpoints derived from two-dimensional (glass plate or leaf disc) studies. A dilution factor of 10 is recommended by ESCORT 2. For 3-dimensional studies, *i.e.* where spray treatment is applied onto whole plants, the dilution factor of 10 is not used, as any dilution over the 3-dimensional vegetation surface is accounted for in the study design.

The predicted exposure rates for off-field exposure ($PER_{off-field}$) have been calculated according to ESCORT II and summarised in the table below. The default distance of 1 m for field crops has been considered in the calculation of the PER, both with and without a vegetation distribution factor (to be used in conjunction with two dimensional and three dimensional exposure test data, respectively).

Table CP 10.3.2-5 PER for off-field exposure following the uses of Prothioconazole + Spiroxamine EC 460

Crop	App. rate (L product/ha)	PER (foliar) (L product/ha)	% Drift (percentile)	Drift factor (% drift/100)	Vegetation distribution factor (VDF)	$PER_{off-field}$ (L product/ha)	
						without VDF [1]	with VDF [2]
Cereals	1 x 1.25	1.25	2.77 (90th)	0.0277	10	0.0346	0.00346
	2 x 1.25	2.13	2.38 (82nd)	0.0238		0.0507	0.00507

[1] For lab test endpoints obtained with 3-D exposure, directly comparable to the distribution of spray drift deposit in 3-D vegetated off-field environment

[2] With dilution factor of 10 for lab test endpoints obtained with 2-D exposure, to adjust for distribution of spray drift deposit in 3-D vegetated off-field environment

The Tier II off-field risk assessments for one and two applications of 1.25 L product/ha to cereals are presented in Table 10.3.2-6 and Table 10.3.2-7, respectively.

Table CP 10.3.2-6 Tier II off-field risk assessment for the proposed use of Prothioconazole + Spiroxamine EC 460 in cereals (1 x 1.25 L product/ha)

Intended use	Cereals			
Product	Prothioconazole + Spiroxamine EC 460			
Application rate (L product/ha)	1 x 1.25			
MAF	1.0			
Species	**LR_{50}/ER_{50} (L product/ha)**	**$PER_{off-field}$ (L product/ha)**	**Corrected $PER_{off-field}$[2] (L product/ha)**	**<50% effects at predicted rate?**
Aphidius rhopalosiphi	>1.25	0.0346[1]	0.173	Y
Aphidius rhopalosiphi	>1.25 (2 x apps)	0.0346[1]	0.173	Y
Aphidius rhopalosiphi	>1.25 (3 x apps)	0.0346[1]	0.173	Y
Typhlodromus pyri	>2.5	0.00346	0.0173	Y

Typhlodromus pyri	>1.25 (2 x apps)	0.0346[1]	0.173	Y
Coccinella septempunctata	>2.875	0.00346	0.0173	Y
Aleochara bilineata	>2.5	0.00346	0.0173	Y

MAF: Multiple application factor; PER: Predicted environmental rate
[1] Study incorporated 3-Dimensional exposure therefore no vegetation distribution factor (VDF) applied
[2] Correction factor of 5 applied for use with extended laboratory data to account for the inter-species variability in sensitivity (ESCORT 2)

Table CP 10.3.2-7 Tier II off-field risk assessment for the proposed use of Prothioconazole + Spiroxamine EC 460 in cereals (2 x 1.25 L product/ha)

Intended use	Cereals			
Product	Prothioconazole + Spiroxamine EC 460			
Application rate (L product/ha)	2 × 1.25			
MAF	1.7 (foliar)			
Species	**LR_{50}/ER_{50} (L product/ha)**	**$PER_{off\text{-}field}$ (L product/ha)**	**Corrected $PER_{off\text{-}field}$[2] (L product/ha)**	**<50% effects at predicted rate?**
Aphidius rhopalosiphi	>1.25	0.0507[1]	0.254	Y
Aphidius rhopalosiphi	>1.25 (2 x apps)	0.0507[1]	0.254	Y
Aphidius rhopalosiphi	>1.25 (3 x apps)	0.0507[1]	0.254	Y
Typhlodromus pyri	>2.5	0.00507	0.0254	Y
Typhlodromus pyri	>1.25 (2 x apps)	0.0507[1]	0.254	Y
Coccinella septempunctata	>2.875	0.00507	0.0254	Y
Aleochara bilineata	>2.5	0.00507	0.0254	Y

MAF: Multiple application factor; PER: Predicted environmental rate
[1] Study incorporated 3-Dimensional exposure therefore no vegetation distribution factor (VDF) applied
[2] Correction factor of 5 applied for use with extended laboratory data to account for the inter-species variability in sensitivity (ESCORT 2)

For both a single and two applications to cereals it is clear that there are less than 50% effects at the $PER_{off\text{-}field}$ with all LR_{50} and ER_{50} values at least >1.25 L product/ha. The off-field risk to NTA populations is therefore acceptable following the proposed uses of Prothioconazole + Spiroxamine EC 460 to cereals.

Biodiversity

No relevant scientifically peer-reviewed open literature could be found on spiroxamine or its major metabolites, from an ecotoxicological perspective, on non-target arthropods (other than bees). Therefore, it is considered that the potential impact of the active substance on biodiversity and the ecosystem, including potential indirect effects *via* alteration of the food web, are covered by the risk assessment for non-target arthropods (other than bees) in this section.

With respect to the NTA in-field and NTA off-field risk assessments, which demonstrated acceptable in-field and off-field risks at the Tier 2 level without the need for risk mitigation, the applicant concludes that the use of the representative lead formulation (Prothioconazole + Spiroxamine EC 460) has a low potential to cause unacceptable effects on biodiversity and the ecosystem *via* trophic interactions. To the best of our knowledge and with the presented safety profile of the spiroxamine a.s. and the representative lead formulation, the applicant does not foresee any unacceptable effects on biodiversity and the ecosystem with spiroxamine.

CP 10.3.2.1 Standard laboratory testing for non-target arthropods

No Tier I data are available for Prothioconazole & Spiroxamine EC 460. However, extended test data are available and have been presented in Section CP 10.3.2.2.

CP 10.3.2.2 Extended laboratory testing, aged residue studies with non-target arthropods

Data Point:	KCP 10.3.2.2/01
Report Author:	[redacted]
Report Year:	2002
Report Title:	Toxicity of JAU 6476 & Spiroxamine EC 460 (JAU 6476 & KWG 4168 EC 460) to predatory mite Typhlodromus pyri Scheuten under extended laboratory conditions
Report No:	011048031
Document No:	M-059177-01-1
Guideline(s) followed in study:	IOBC (Blümel et al. 2000) ESCORT recommendation (Candolfi et al. 2001)
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and accepted RAR (2010)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

A 14-day study was conducted in order to assess the effects of exposure to JAU 6476 & Spiroxamine EC 460 on mortality and reproduction in predatory mites, *Typhlodromus pyri*.

The mites were exposed to bean leaves which had been sprayed at 1.25 or 2.5 L/ha of JAU 6476 & Spiroxamine EC 460 or a control or reference product. Mortality was assessed after 7 days and reproduction after 14 days.

The study showed no statistically significant effects on mortality or reproduction of predatory mites, *Typhlodromus pyri*, when exposed to dried residues of JAU 6476 & Spiroxamine EC 460. The LR_{50} and ER_{50} were therefore considered to be >2.5 L product/ha.

I. Materials and Methods

Materials

Test Material	JAU 6476 & Spiroxamine EC 460 (JAU 6476 & KWG 4168 EC 460)
Lot/Batch #:	06920/0045(0019)
Purity:	JAU 6476: 155.47 g/L KWG 4168: 303.0 g/L
Description:	Clear brown liquid
Stability of test compound:	Not reported
Reanalysis/Expiry date:	15 May 2001

Density:	0.984 g/cm³
Treatments	
Test rates:	1.25 and 2.5 L product/ha
Solvent/vehicle:	Deionised water
Analysis of test concentrations:	Applied rates were 102 - 105% of nominal
Test organisms	
Species:	Predatory mite, *Typhlodromus pyri* (SCHEUTEN), Acari: Phytoseiidae
Source:	PK Nutzlingszuchten, D-73642 Welzheim
Feeding:	Pine (*Pinus negra*) and birch (*Betula pendula*) pollen on each assessment day
Treatment for disease:	None reported.
Test design	
Test vessel:	Bean leaf disc on moistened cotton wool in a Petri dish (9 cm)
Replication:	5 per treatment group
No. animals/vessel:	20
Duration of test:	14 days
Environmental test conditions	
Temperature:	24 - 26°C
Photoperiod:	16 hour light : 8 hour dark, 2000 lux

Study Design

This study was conducted in order to assess the effects of JAU 6476 & Spiroxamine EC 460 on mortality and reproduction in predatory mites (*Typhlodromus pyri*) over 14 days.

The test item was sprayed onto the leaves of a kidney bean plant (*Phaseolus vulgaris*). The control was 200 L/ha deionised water, the reference item was Dimethoate EC 400 at a concentration of 10 mL/ha in 200 L/ha water. The test item of JAU 6476 & Spiroxamine EC 460 was applied at concentrations of 2.5 L product/ha and 1.25 L product/ha in 200 L/ha of water.

Spray fluid was automatically applied to the ventral leaf surface to ensure uniform deposition of the spray onto the bean leaves. The solutions were applied at 200 L/ha (2 mg/cm²) ± 10%. The applied rates ranged from 102 to 105% of the nominal.

After the sprayed residues had dried, 5 replicates of 20 mites per treatment group were placed on the treated bean leaf discs situated on moistened cotton wool in a Petri dish (9 cm). The mites used in the study were 1-day-old protonymphs from a synchronised cohort.

On each assessment day, the mites were fed with pine or birch pollen. Temperature during the study was maintained at 24 to 26°C and the photoperiod was 16 hours of light at 2000 lux. These environmental conditions were continuously recorded.

On day 3, 7, 9, 11 and 14 after the application, the number of surviving predatory mites were counted and on days 9, 11 and 14 the number of laid eggs was determined. The final assessment for the mortality

was performed on day 7 after treatment and the final assessment for reproduction was made on day 14 after treatment.

II. Results and Discussion

Validity criteria according to the guideline that the study was conducted to, Blümel *et al.* (2000), were met.

- Mean mortality in the control ≤20% on day 7 (actual: 5%)
- Cumulative mean number of eggs per female in the control from day 7 to 14 ≥4 (actual: 4.45 eggs/female)
- Mean mortality in the reference item on day 7 is between 50% and 100% (actual: 62%)

There were no statistically significant differences in mortality and reproduction observed in the treatment group when compared to the control group. No behavioural anomalies were recorded in all treatment groups.

After 7 days, mortality in the highest test item concentration group, 2.5 L product/ha, was 11% and the number of eggs/female was reduced by 6.5% relative to the control.

Table CP 10.3.2.2/01-1 Mortality and reproduction of Typhlodromus pyri after exposure to JAU 6476 & Spiroxamine EC 460

Test item treatment group (L/ha)	Mortality after 7 days (%)	Mean number eggs/female after 14 days	Reduction in number eggs/female relative to control (%)
Control	5	4.45	-
1.25	7	4.48	0
2.5	11	4.16	6.5
Reference	62	-	-

- not assessed

III. Conclusion

The predatory mite *Typhlodromus pyri* was exposed to bean leaf discs treated at rates of 1.25 or 2.5 L/ha of JAU 6476 & Spiroxamine EC 460 or a control or reference product. Mortality was assessed after 7 days and reproduction after 14 days.

The study showed no statistically significant effects on mortality or reproduction of *T. pyri*, when exposed to dried residues of JAU 6476 & Spiroxamine EC 460. The LR_{50} and ER_{50} were therefore considered to be >2.5 L product/ha.

Assessment and conclusion by applicant:

Validity criteria according to the current test guideline method by Blümel *et al.* (2000) were met.

- Mean mortality in the control ≤20% on day 7 (actual: 5%)
- Cumulative mean number of eggs per female in the control from day 7 to 14 ≥4 (actual: 4.45 eggs/female)
- Mean mortality in the reference item on day 7 is between 50% and 100% (actual: 62%)

The study has been conducted to the most recent IOBC test method and has followed the recommended methods and procedures. The study is therefore considered to be acceptable.

The LR_{50} and ER_{50} were considered to be >2.5 L product/ha.

Data Point:	KCP 10.3.2.2/02
Report Author:	[redacted]
Report Year:	2002
Report Title:	Toxicity of JAU 6476 & spiroxamine EC 460 (JAU 6476 & KWG 4168 EC 460) to the predatory mite Typhlodromus pyri Scheuten under extended laboratory conditions (aged-residue test)
Report No:	011048032
Document No:	M-059404-01-1
Guideline(s) followed in study:	IOBC (Blümel et al. 2000) ESCORT recommendation (Barrett et al. 1994)
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and accepted RAR (2010)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

A 14-day study was conducted in order to assess the effects of JAU 6476 & Spiroxamine EC 460 on mortality and reproduction in predatory mites, *Typhlodromus pyri*.

The mites were exposed to a control and reference product and to maize plants treated with two applications (21-day interval) of 1.25 L/ha of JAU 6476 & Spiroxamine EC 460. Mites were exposed to the test item following the last application of the test item and 7 days after the last application. Mortality was assessed after 7 days of exposure and reproduction after 14 days.

The study showed no statistically significant effects on mortality or reproduction of predatory mites, *Typhlodromus pyri*, when exposed to dried residues JAU 6476 & Spiroxamine EC 460. The LR_{50} and ER_{50} were therefore considered to be >2 x 1.25 L product/ha at both 0 and 7 days after treatment.

I. Materials and Methods

Materials

Test Material JAU 6476 & Spiroxamine EC 460 (JAU 6476 & KWG 4168 EC 460)

Lot/Batch #:	06920/0045(0019)
Purity:	JAU 6476: 160.4 g/L KWG 4168: 296.2 g/L
Description:	Clear dark yellow liquid
Stability of test compound:	Not reported
Reanalysis/Expiry date:	2 November 2001
Density:	0.984 g/cm^3

Treatments

Test rates:	2 x 1.25 L product/ha
Solvent/vehicle:	Water
Analysis of test concentrations:	The applied rates ranged from 104 to 109% of the nominal rates.

Test organisms

Species:	One day old predatory mite, *Typhlodromus pyri* (SCHEUTEN), Acari: Phytoseiidae of a synchronised cohort
Source:	PK Nutzlingszuchten, D-73642 Welzheim
Feeding:	Pollen: pine (*Pinus negra*) and birch (*Betula pendula*) 1:1, at each assessment day
Treatment for disease:	None reported

Test design

Test vessel:	Maize leaf pieces (1.5 x 4 cm) on moistened cotton wool in a Petri dish of diameter 0 9 cm
Replication:	5 per treatment group
No. animals/vessel:	20
Duration of test:	14 days

Environmental test conditions

Temperature:	24 - 27°C
Photoperiod:	16 hour light : 8 hour dark, 2000 lux

Study Design

This study was conducted in order to assess the effects of JAU 6476 & Spiroxamine EC 460 on mortality and reproduction in predatory mites (*Typhlodromus pyri*) over 14 days.

The control was 200 L/ha deionised water, the reference item was Dimethoate EC 400 at a concentration of 10 mL/ha in 200 L/ha water. The test item of JAU 6476 & Spiroxamine EC 460 was applied twice at a concentration 1.25 L product/ha in 200L/ha of water. The second application was sprayed 21 days after the first.

The test item was sprayed onto potted maize plants using a plot-sprayer. The solutions were applied at 200 L/ha ± 10%. The applied rates ranged from 104 to 109% of the nominal rates.

After the sprayed residues had dried, five replicates of 20 mites per treatment group were exposed to treated maize leaf discs on the upper leaf surface. Another group of mites were exposed to the test item for a second time 7 days later. The mites used in the study were 1-day-old protonymphs from a synchronised cohort.

On each assessment day, the mites were fed with pine and birch pollen. Temperature during the study was maintained at 24 to 27°C and the photoperiod was 16 hours of light at 2000 lux. These environmental conditions were continuously recorded.

On Day 3, 7, 9, 11 and 14 after the application, the number of surviving predatory mites were counted and on Days 9, 11 and 14 the number of laid eggs was determined. The final assessment for the mortality was performed on Day 7 after treatment and the final assessment for reproduction was made on Day 14 after treatment.

II. Results and Discussion

Validity criteria according to the test method to which the study was conducted, Blümel *et al.* (2000), were met.

- Mean mortality in the control ≤20% on day 7 (actual: 5 and 4% in the first and second exposure, respectively)
- Cumulative mean number of eggs per female in the control from day 7 to 14, ≥ 4 eggs per female (actual: 7.50 and 7.01 eggs/female in the first and second exposure, respectively)
- Mean mortality in the reference item on day 7 is between 50% and 100% (actual: 87 and 84% in the first and second exposure, respectively)

In the first exposure bioassay, there were no statistically significant differences in mortality and reproduction observed in the treatment group when compared to the control group. Reproduction in the test item treatment group was greater than reproduction observed in the control group.

Table CP 10.3.2.2/02-1 First exposure - mortality and reproduction of mites after exposure to test item

Test item treatment group (L/ha)	Mortality after 7 days exposure (%)	Mean number eggs/female after 14 days exposure	Number eggs/female relative to control (%)
Control	5	7.50	
2 x 1.25	4	7.52	100.3
Reference	87	-	

- not assessed

In the mites exposed to test item residues in the second exposure bioassay (7 days after the first exposure), reproduction was at 98.6% of that observed in the control. There was no statistically significant difference in the mortality observed in the test item group and the control.

Table CP 10.3.2.2/02-2 Second exposure - mortality and reproduction of mites after exposure to test item

Test item treatment group (L/ha)	Mortality after 7 days exposure (%)	Mean number eggs/female after 14 days exposure	Number eggs/female relative to control (%)
Control	4	7.01	
2 x 1.25	6	6.91	98.6
Reference	84		

- not assessed

III. Conclusion

The predatory mite *Typhlodromus pyri* was exposed to maize leaf discs on which had been sprayed 1.25 L/ha of JAU 6476 & Spiroxamine EC 460 (2 x applications) or a control or reference product. Mortality was assessed after 7 days and reproduction after 14 days in two bioassays at 0 and 7 days after treatment.

The study showed no statistically significant effects on mortality or reproduction of *T. pyri*, when exposed to fresh dried or 7-day aged residues of JAU 6476 & Spiroxamine EC 460. The LR_{50} and ER_{50} were therefore considered to be >2 x 1.25 L product/ha at both 0 and 7 days after treatment.

Assessment and conclusion by applicant:

The study was conducted to the most recent IOBC test method by Blümel *et al.* (2000) and the validity criteria were met:

- Mean mortality in the control ≤20% on day 7 (actual: 5 and 4% in the first and second exposure, respectively)
- Cumulative mean number of eggs per female in the control from day 7 to 14, ≥ 4 eggs per female (actual: 7.50 and 7.01 eggs/female in the first and second exposure, respectively)

- Mean mortality in the reference item on day 7 is between 50% and 100% (actual: 87and 84% in the first and second exposure, respectively)

The study is therefore considered acceptable. Although an aged residues test, the fresh residues bioassay (*i.e.* 0 DAT) demonstrated <50% effects on mortality and reproduction. An LR_{50} and ER_{50} value of >2 x 1.25 L product/ha can therefore be determined and used in the risk assessment.

Data Point:	KCP 10.3.2.2/03
Report Author:	[redacted]
Report Year:	2002
Report Title:	Acute toxicity of JAU 6476 & spiroxamine EC 460 (JAU 6476 & KWG 4168 EC 460) to the cereal aphid parasitoid Aphidius rhopalosiphi (Destefani-Perez) under extended laboratory conditions
Report No:	011048029
Document No:	M-065998-01-1
Guideline(s) followed in study:	IOBC guideline proposal (Mead-Briggs & Longley 1997), IOBC guideline (Mead-Briggs et al. 2000) ESCORT 2 recommendation (Candolfi et al. 2001)
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and accepted RAR (2010)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

Aphidius rhopalosiphi were exposed to JAU 6476 & Spiroxamine EC 460 under extended laboratory conditions to assess the effects on mortality and reproduction.

The test item was applied at rates of 1.25 and 2.5 L product/ha in 200 L deionised water/ha to maize leaves. The control was treated with deionised water (200 L/ha) and Dimethoate EC 400 (10 mL product/ha in 200 L/ha of water) was used as a toxic reference treatment.

Exposure to JAU 6476 & Spiroxamine EC 460 did not cause any significant impacts on mortality and reproduction when compared to the control treatment group at a rate of 1.25 L product/ha. At 2.5 L product/ha there was 100% mortality of the wasps. The LR_{50} and ER_{50} were therefore estimated to be >1.25 L product/ha.

I. Materials and Methods

Materials

Test Material JAU 6476 & Spiroxamine EC 460
(JAU 6476 & KWG 4168 EC 460)

Lot/Batch #: 06920/0045(0019)

Purity: JAU 6476: 155.47g/L
KWG 4168: 303.07 g/L

Description: Clear brown liquid

Stability of test compound: Not reported

Reanalysis/Expiry date:	15 May 2002
Density:	0.984 g/cm³
Treatments	
Test rates:	1.25 and 2.5 L product/ha
Solvent/vehicle:	Deionised water
Analysis of test concentrations:	Application rates varied within 102-103% of nominal rates.
Test organisms	
Species:	Adult cereal aphid parasitoid, *Aphidius rhopalosiphi*
Source:	PK Nutzlingszuchten, D-73642 Welzheim
Acclimatisation period:	None
Feeding:	Mortality test: aqueous fructose solution (25% w/v) was applied on the test plants 1 hour before application of test item
Treatment for disease:	None reported
Test design	
Test vessel:	Mortality test: 2 square glass plates (13 x 13cm), held apart by an aluminium frame (13 x 13 x 1.4 cm) with gauze covered holes for forced air ventilation (blowing air; flow rate: 2.5 L/min) Parasitisation test: acrylic cylinder (11 cm diameter, 20 cm high) with 20 wheat seedlings (8 days old) infested with >100 aphids (second to third instar) and covered at the top of the cylinder with gauze.
Replication:	Mortality test: 4 Parasitisation test: 15
No. animals/vessel:	Mortality test: 5 Parasitisation test: 1
Duration of test:	Mortality test: 48 hours Parasitisation test: 11 days
Environmental test conditions	
Temperature:	20 - 22°C
Relative humidity:	60 - 67%
Photoperiod:	16 hour light : 8 hour dark 2000 lux (mortality phase), 4100 lux (parasitisation test)

Study Design

The parasitic wasp, *Aphidius rhopalosiphi* was exposed to JAU 6476 & Spiroxamine EC 460 under extended laboratory conditions after residual contact exposure on maize plants over 48 hours to assess mortality. Effects on reproduction were assessed over 10 days following the mortality assessment.

Wasps had undergone metamorphosis less than 48 hours prior to being used in the test. Adult wasps were exposed in four replicates of five wasps per treatment group to the residues of the test item, reference item and control.

The wasps were not fed but only watered for 12 to 18 hours prior to exposure and during the assessments the wasps were fed with aqueous fructose solution (25% w/v).

The test items were applied to potted maize plants at rates of 1.25 and 2.5 L product/ha in 200 L deionised water/ha. The control group was treated with deionised water (200 L/ha) and Dimethoate EC 400 (10 mL product/ha in 200 L/ha of water) was used as a toxic reference treatment. The test solution was sprayed onto leaves using an automatic application cabin. Actual applied rates varied from 102 to 105% of target spray volume.

Following the air-drying of the spray deposit (about 1 hour), treated maize leaves were laid on the bottom glass plate of the test vessel for the mortality phase. Test organisms were added to the test vessel that was closed with dark material pervious to air and placed in a climatic test room. The temperature was 20 to 22°C and was continuously measured.

Five female wasps were confined to each cage and at 1, 2, 24 and 48 hours after exposure, the number of surviving wasps and their condition were determined. Distinction was drawn between alive, affected, moribund and dead animals. Thirty minutes and two hours after exposure, the behaviour of the wasps were recorded at 3-minute intervals as either on the plant, on the untreated sidewalls, or on the untreated top glass plate over a 30-minute period.

After 48 hours, to determine the parasitisation capacity, 15 of the surviving females of the control group and the treated groups were randomly selected and individually confined in acrylic cylinders containing untreated potted wheat plants infested with more than 100 nymphal aphids. The wasps were removed 24 hours later and the parasitisation vessels were stored in the climatic room for a further 10 days. The number of parasitised aphids was recorded and the parasitisation rate per wasp was determined.

II. Results and Discussion

Validity criteria according to the test method to which the study was conducted, Mead-Briggs *et al.* (2000), were met.

- Mortality in the control group ≤12.5% (actual: 5%)
- Mortality in the reference item group ≥50% (actual: 100%)
- Mummies per female produced in the control group ≥5 (actual: 13.1)
- Number of female wasps producing no mummies ≤2 (actual: 1)

In the 2.5 L product/ha test item group and the reference item group 100% mortality was observed. There was no significant difference between the 1.25 L product/ha test item group and the control group in the mean number of mummies produced per female.

Table CP 10.3.2.2/03-1 Mortality and fecundity of Aphidius rhopalosiphi after 48 hours exposure to test item

Test item concentration (L/ha)	Mortality (%)	Mean no. mummies/female
Control	5	13.1
1.25	5	13.5
2.5	100	-
Reference	100	-

- not determined

The behaviour assessments showed statistically significant differences compared to the control group after 30 minutes in both test item treatment groups and in the higher dosed test item group after 2 hours of exposure.

The reproductive performance at the 1.25 L product/ha treatment relative to control was 103%.

Based on these results the LR_{50} and ER_{50} were estimated to be >1.25 L product/ha.

III. Conclusion

The parasitic wasp *Aphidius rhopalosiphi* was exposed to JAU 6476 & Spiroxamine EC 460 applied to maize leaves at rates of 1.25 and 2.5 L product/ha in order to assess the effects of exposure on mortality and fecundity.

Exposure to JAU 6476 & Spiroxamine EC 460 did not cause any significant impacts on mortality and reproduction when compared to the control treatment group at a rate of 1.25 L product/ha. At 2.5 L product/ha there was 100% mortality of the wasps. The LR_{50} and ER_{50} were therefore estimated to be >1.25 L product/ha.

Assessment and conclusion by applicant:

The study was conducted to the current IOBC test method guideline by Mead-Briggs *et al.* (2000) and the validity criteria were met:

- Mortality in the control group ≤12.5% (actual: 5%)
- Mortality in the reference item group ≥50% (actual: 100%)
- Mummies per female produced in the control group ≥5 (actual: 13.1)
- Number of female wasps producing no mummies ≤2 (actual: 1)

The study is therefore considered acceptable.

The LR_{50} and ER_{50} were estimated to be >1.25 L product/ha.

Data Point:	KCP 10.3.2.2/04
Report Author:	[redacted]
Report Year:	2005
Report Title:	Effects of prothioconazole & spiroxamine EC 160 + 300 on the parasitoid Aphidius rhopalosiphi, extended laboratory study - aged residue test -
Report No:	26221003
Document No:	M-259098-01-1
Guideline(s) followed in study:	(GLP compliant study based on Mead-Briggs et al. 2000 modified for the exposure on natural substrate according to the recommendations of Mead-Briggs et al. 2002
Deviations from current test guideline:	Yes During the parasitisation period temperature was higher than 22 °C (maximum 24 °C) for approximately 4 hours Third application: application rate was more than 10 % greater than the target amount (+16.3 %) Deviations had no effect on the study
Previous evaluation:	yes, evaluated and accepted RAR (2010)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The objective of this study was to determine the effects of Prothioconazole & Spiroxamine EC 160 + 300 exposure to the parasitoid *Aphidius rhopalosiphi* in the laboratory by contacting fresh and aged spray residues on plant surfaces, compared to a water treated control and to a reference item.

There were no effects of Prothioconazole & Spiroxamine EC 160 + 300 on mortality and reproduction of *A. rhopalosiphi* when exposed to freshly dried residues on barley plants on the day of the 3rd application (1st bioassay) and to aged residues on barley plants 7 days after the 3rd application (2nd bioassay).

In both, the 1st and 2nd bioassay there were no repellent effects of the test item observed when compared to the control.

The LR_{50} and ER_{50} were considered to be > 3 x 1.25 L product/ha.

I. Materials and Methods

Materials

Test Material Prothioconazole & Spiroxamine EC 160 + 300

Lot/Batch #: 06920/0109(0019)

Purity: JAU 6476: 160.23 g/L
KWG 4168: 292.93 g/L

Description: Clear dark brown liquid

Stability of test compound: Considered stable until expiration date

Reanalysis/Expiry date: 22 December 2005

Density: 0.983 g/mL

Treatments

Test rates: 3 x 1.25 L product/ha

Solvent/vehicle:	Tap water
Analysis of test concentrations:	None

Test organisms

Species:	*Aphidius rhopalosiphi*
Source:	Katz Biotech AG, An der Birkenpfuhlheide 10, D-15837 Baruth, Germany
Feeding:	10 % fructose solution

Test design

Test vessel:	*Hatching chambers:* Glass tubes with a length of approximately 15 cm and a diameter of 1.5 cm at the large and 0.5 cm at the small opening. *Exposure units:* Suitably-sized pots with 4 - 5 treated barley seedlings (*Hordeum vulgare* 'Xanadu') planted in the middle of each pot were used. *Post-exposure units* (parasitisation and post-parasitisation period): Untreated pots (13 cm in diameter) with barley seedlings (*Hordeum vulgare* 'Duett', 10 - 25 seedlings, 9 days old) infested with approximately 150 - 200 host aphids (estimated number) of all developmental stages (*Rhopalosiphum padi*) were enclosed within a clear polyacrylic cylinder (30 cm high and 10 cm in diameter).
Replication:	6 replicates per treatment and control group for the exposure period and 20 replicates per treatment and control for post-exposure.
No. animals/vessel:	5 females for exposure period and 1 female per replicate for the post-exposure period
Duration of test:	48 hours + 24 hours

Environmental test conditions

Temperature:	18 - 24°C
Relative humidity:	60 – 90% (acclimation, exposure, parasitisation period) 62 – 63% (post-parasitisation period; within the test units)
Lighting:	800 – 2700 lux (acclimation, exposure, parasitisation period) 6000 – 17400 lux (post-parasitisation period)

Study Design

The objective of this study was to determine the toxic effects of Prothioconazole & Spiroxamine EC 160 + 300 on the parasitoid *Aphidius rhopalosiphi* in the laboratory by contacting fresh and aged spray residues on plant surfaces, compared to a water treated control and to a reference item. Additionally, an assessment for sublethal effects on parasitisation activity of the female survivors was made.

The test species were obtained as aphid mummies from Katz Biotech AG, An der Birkenpfuhlheide 10, D-15837 Baruth. Acclimatisation was approximately two days under test conditions in hatching chambers.

The hatching chambers were glass tubes with a length of approximately 15 cm and a diameter of 1.5 cm at the large and 0.5 cm at the small opening. The exposure units were suitably sized pots with four to five treated barley seedlings planted in the middle of each pot. On the day of the of the 1st application the plants of all treatment groups (control, test item and reference item) were at the 2nd to 4th leaf growth

stage. *i.e.* BBCH Growth stage 12 - 14. The plants were trimmed to a uniform height of 10 cm tall on the day of the 1st application prior application. Treated leaves were not cut again to make sure that no treated area of the plants was getting lost. New grown and untreated leaves were trimmed to the same size as the treated leaves of the plants before the 2nd and 3rd application (the day of the 1st bioassay) and before the 2nd bioassay. The plants were enclosed within a clear polyacrylic cylinder (45 cm high and 15 cm in diameter) with a hole (approximately 1.5 - 2.1 cm in diameter) for the introduction of the parasitoids. After introduction the hole was closed by a stopper with a hole where the ventilation tube was inserted. The opening of ventilation tube was closed with a fine mesh gauze. The top of the cylinder was closed with a fine mesh gauze. The soil surface was covered with a thin layer of quartz sand before each bioassay.

Hatching chambers consisted of glass tubes with a length of approximately 15 cm and a diameter of 1.5 cm at the large and 0.5 cm at the small opening.

Exposure units were suitably-sized pots with 4 - 5 treated barley seedlings (*Hordeum vulgare* 'Xanadu') planted in the middle of each pot were used. On the day of the 1st application the plants of all treatment groups (control, test item, reference item) were at the 2nd - 4th leaf growth stage, i.e. BBCH Growth Stage 12 - 14. The plants were trimmed to a uniform height of 10 cm tall on the day of the 1st application prior application. Treated leaves were not cut again to make sure that no treated area of the plants was getting lost. New grown and untreated leaves were trimmed to the same size as the treated leaves of the plants before the 2nd and 3rd application (the day of the 1st bioassay) and before the 2nd bioassay. The plants were enclosed within a clear polyacrylic cylinder (45 cm high and 15 cm in diameter) with a hole (approximately 1.5 - 2.1 cm in diameter) for the introduction of the parasitoids. After introduction the hole was closed by a stopper with a hole where the ventilation tube was inserted. The opening of ventilation tube was closed with a fine mesh gauze. The top of the cylinder was closed with a fine mesh gauze. The soil surface was covered with a thin layer of quartz sand before each bioassay.

Post-exposure units used for the parasitisation and post-parasitisation period were untreated pots (13 cm in diameter) with barley seedlings (*Hordeum vulgare* 'Duett'; 10 - 25 seedlings, 9 days old) infested with approximately 150 - 200 host aphids (estimated number) of all developmental stages (*Rhopalosiphum padi*) were enclosed within a clear polyacrylic cylinder (30 cm high and 10 cm in diameter). The cylinder had two holes (70 x 195 mm) which were closed with a fine gauze to improve the ventilation and another hole (approximately 1.5 - 2.1 cm in diameter) closed with cotton wool for the introduction of the parasitoids. The top of the cylinder was also covered with a fine gauze. The soil surface was covered with a thin layer of quartz sand.

Treatments were applied to barley plants outdoors under field conditions and maintained outdoors under semi-field conditions. Exposure of the adults of the parasitoids occurred on pre-treated plants and maintained in the laboratory under controlled conditions.

The climatic conditions between the three applications of the test substance in terms of temperature and relative humidity were 9 to 36°C and 30 to 100% relative humidity. During the aging of the residues the temperatures were 9 to 26°C and the relative humidity was 38 to 100%.

The treated plants were protected against rain with a thin plastic sheeting between the applications and following the applications up to 8 days after the last application. There was heavy rain fall on day 7 and therefore rain protection was removed on day 8 instead of day 7 after the 3rd application.

A 10% fructose solution was provided on a cotton wool pad during acclimatisation, *ad libitum*. During the exposure period, 25 minutes to 60 minutes before each bioassay, the treated seedlings were lightly sprayed with the sugar solutions and left to dry.

The application in the field consisted of three applications according to agricultural practice with a movable plot sprayer for field application, type TSG with an extension tube including 4 spraying nozzles (TeeJet DG 11002; distance between nozzles first and third application: 50 cm, second application: 100 cm, spraying pressure: 3.0 bar). The concentration of the test substance spraying dilution for the first, second and third application was 9.22 g product in 3 L tap water (3.07 g product/L).

The concentration of the reference item spraying dilution for the first and second application was 75 µL Perfekthion in 3 L tap water (corresponding to 25 µL Perfekthion/L). application was conducted in the laboratory using a calibrated laboratory spraying equipment (Fa. Schachtner, D-71640 Ludwigsburg).

During the first and second application, the test substance was applied, during the third application the control, test substance and reference item were applied.

Applications and aging of the test item were done in the field under natural conditions. In each bioassay *A. rhopalosiphi* was exposed to the treated barley plants after aging of a determined time. The first bioassay was carried out with freshly dried residues (50 - 55 minutes after the 3rd application). The second bioassay on aged residues was started on day 7 after the third application. Due to the low effects of Prothioconazole & Spiroxamine EC 160 + 300 on mortality and reproduction of *A. rhopalosiphi* in the first and second bioassay it was not necessary to make further bioassays.

During the first bioassay, individuals were introduced after drying of the spray layer and the food, 50 to 55 minutes after application (control, test substance and reference substance). Whereas, during the second bioassay individuals were introduced on day seven after the third application after drying of the food (control and test substance) after drying of the spray layer and the food, 55 minutes after application. Individuals were impartially introduced and transfer was done using an aspirator, following the spraying scheme; only live and apparently unaffected parasitoids were introduced into the exposure units.

The exposure time was approximately 48 hours. The post-exposure time for the parasitisation period was 24 hours and the post-parasitisation period was 11 days.

Observations of mortality were recorded approximately 2, 24 and 48 hours after test initiation. The numbers of parasitoids alive, affected, moribund and dead were recorded. Moribund parasitoids were counted as dead. To determine whether residues of the test item are repellent to the wasps, observations on the position of the individual insects were made during the initial 3 hours after their release. Five separate observations were made at approximately 30-minute intervals starting approximately 30 minutes after the introduction of all wasps. Each wasp was recorded as being on the plants, cylinder or soil.

Observations of reproduction were conducted 11 days after the 24 hour parasitisation period in all replicates where the females were alive after the 24 hours parasitisation period, by counting the mummies. Reproduction was performed where the corrected mortality was ≤ 50%. No reproduction testing was performed with the reference substance.

The experiment was performed in a controlled environment room at a temperature of 18 - 24°C (deviation from study plan) and a relative humidity of 60 - 90%. The light intensity was dark cycle was 16:8 hours. The light intensity was 800 - 2700 lux in the exposure and parasitisation period and 6000 – 17400 lux in the post-parasitisation period. The light/dark cycle was 16 hour light : 8 hour dark.

II. Results and Discussion

Validity criteria according to the study report were met:

- Control mortality to not exceed 17% (actual: 6.7 and 3.3% in the 1st and 2nd bioassay, respectively)
- Reference item mortality to be at least 50% (actual: 92.9 and 96.6% in the 1st and 2nd bioassay, respectively)
- Control reproduction rate to be ≥5 mummies per female (actual: 29.1 and 34.3 in the 1st and 2nd bioassay, respectively)
- No more than two females should produce no mummies (actual: 0 and 1 females producing no mummies in the 1st and 2nd bioassay, respectively)

Table CP 10.3.2.2/04-1 Percent mortalities of the parasitoids – 1st bioassay: test start on the day of the 3rd application

Treatment groups	Mortality[a] [%]	Corrected mortality [%]
Control	6.7 ± 10.3	-
3 x 1.25 L product/ha	6.7 ± 16.3	0.0
Reference item	93.3 ± 16.3 *	92.9

* Significantly different to the control (Fisher's exact test, α = 0.05)

[a] after 48 hours exposure to the test item residues on plant surfaces; percentage values represent mean and standard deviation from six replicates each with five females.

Table CP 10.3.2.2/04-2 Behavioural observations of the parasitoids – 1st bioassay: test start on the day of the 3rd application

	Control	Test item 3 x 1.25L product/ha	Reference item
Introduced	30	30	30
After 2 hours			
Alive	30	30	30
Affected	0	0	0
Moribund	0	0	0
Dead	0	0	0
After 24 hours			
Alive	30	30	12
Affected	0	0	0
Moribund	0	0	0
Dead	0	0	18
After 48 hours			
Alive	28	28	1
Affected	0	0	1
Moribund	0	0	0
Dead	2	2	28

Table CP 10.3.2.2/04-3 Percentage of *Aphidius rhopalosiphi* on treated plants – 1st bioassay: test start on the day of the 3rd application

Time after test start [h]	Control [%][a]	Test item 3 x 1.25 L product/ha [%][a]	Reference item
0.5	80.0 ± 12.6	73.3 ± 16.3	70.0 ± 21.0
1.0	85.0 ± 17.3	86.7 ± 10.3	83.3 ± 15.1
1.5	73.3 ± 20.7	96.7 ± 8.2	73.3 ± 10.3

Time after test start [h]	Control [%][a]	Test item 3 x 1.25 L product/ha [%][a]	Reference item
2.0	86.7 ± 16.3	76.7 ± 29.4	66.7 ± 16.3
2.5	80.0 ± 12.6	80.0 ± 17.9	86.7 ± 10.3
Total	81.0 ± 8.1	82.7 ± 14.0 n.s.	76.0 ± 6.7 n.s.

[Student-t-Test, α = 0.05; n.s. = not significant, * = significant]

[a] percentage values represent means and standard deviation from six replicates each with five females

Table CP 10.3.2.2/04-4 Parasitisation efficiency of the parasitoids – 1st bioassay: test start on the day of the 3rd application

Treatment groups	Parasitisation rate[a] [mummies per female]	Reduction of parasitisation efficiency [%]
Control	29.1 ± 14.0	-
3 x 1.25 L product/ha	23.2 ± 16.4 n.s.	13.4

[Student-t-Test, α = 0.05; n.s. = not significant]

[a] parasitoids previously exposed to the test item residues on plant surfaces; values represent means and standard deviation from maximum twenty replicates each with one females

Table CP 10.3.2.2/04-5 Percent mortalities of the parasitoids – 2nd bioassay: test start 7 days after the 3rd application

Treatment groups	Mortality[a] [%]	Corrected mortality [%]
Control	3.3 ± 8.2	-
3 x 1.25 L product/ha	6.7 ± 10.3	3.4
Reference item	96.7 ± 8.2 *	96.6

* Significantly different to the control (Fisher's exact test, α = 0.05)

[a] after 48 hours exposure to the test item residues on plant surfaces; percentage values represent mean and standard deviation from six replicates each with five females.

Table CP 10.3.2.2/04-6 Behavioural observations of the parasitoids – 2nd bioassay: test start seven days after the 3rd application

	Control	Test item 3 x 1.25L product/ha	Reference item
Introduced	30	30	30
After 2 hours			
Alive	30	30	30
Affected	0	0	0
Moribund	0	0	0

	Control	Test item 3 x 1.25L product/ha	Reference item
Dead	0	0	0
After 24 hours			
Alive	30	30	16
Affected	0	0	0
Moribund	0	0	0
Dead	0	0	14
After 48 hours			
Alive	29	28	1
Affected	0	0	0
Moribund	0	0	0
Dead	1	2	29

[a] exposure of parasitoid on treated plant surfaces

[b] summary of six replicates, each replicate contained a total of five female parasitoids

Table CP 10.3.2.2/04-7 Percentage of *Aphidius rhopalosiphi* on the treated plants – 2nd bioassay: test start seven days after the 3rd application

Time after test start [hours]	Control [%][a]	Test item 3 x 1.25 L product/ha [%][a]	Reference item [%][a]
0.5	86.7 ± 16.3	76.7 ± 15.1	80.0 ± 12.6
1.0	86.7 ± 16.3	86.7 ± 10.3	63.3 ± 19.7
1.5	93.3 ± 10.3	86.7 ± 16.3	57.5 ± 21.9
2.0	83.3 ± 15.1	76.7 ± 15.1	80.0 ± 17.9
2.5	83.3 ± 15.1	83.3 ± 15.1	63.3 ± 15.1
Total	86.7 ± 8.3	82.0 ± 11.0 n.s.	68.8 ± 4.3

Student-t-Test, α = 0.05; n.s. = not significant, * = significant]

[a] percentage values represent means and standard deviation from six replicates each with five females

Table CP 10.3.2.2/04-8 Parasitisation efficiency of the parasitoids – 2nd bioassay: test start seven days after the 3rd application

Treatment groups	Parasitisation rate[a] [mummies per female]	Reduction of parasitisation efficiency [%]
Control	34.3 ± 15.9	-
3 x 1.25 L product/ha	37.9 ± 18.6	-10.6
[Student-t-Test, α = 0.05; n.s. = not significant]		

[a] parasitoids previously exposed to the test item residues on plant surfaces; values represent means and standard deviation from maximum twenty replicates each with one female

Treatment groups	Parasitisation rate[a] [mummies per female]	Reduction of parasitisation efficiency [%]

[b] negative value mean increased parasitisation efficiency compared to the control

III. Conclusion

There were no effects of Prothioconazole & Spiroxamine EC 160 + 300 on mortality and reproduction of *A. rhopalosiphi* when exposed to freshly dried residues on barley plants on the day of the 3rd application (1st bioassay) and to aged residues on barley plants 7 days after the 3rd application (2nd bioassay).

In both, the 1st and 2nd bioassay there were no repellent effects of the test item observed when compared to the control.

The LR_{50} and ER_{50} were considered to be >3 x 1.25 L product/ha.

Assessment and conclusion by applicant:

The study has been assessed against the validity criteria according to the current extended *Aphidius* test method guideline by Mead-Briggs *et al.* (2009). The validity criteria were met.

- Control mortality to not exceed 10% (actual: 6.7 and 3.3% in the 1st and 2nd bioassay, respectively)
- Reference item mortality to be at least 50% (actual: 92.9 and 96.6% in the 1st and 2nd bioassay, respectively)
- Control reproduction rate to be ≥5 mummies per female (actual: 29.1 and 34.3 in the 1st and 2nd bioassay, respectively)
- No more than two females should produce no mummies (actual: 0 and 1 females producing no mummies in the 1st and 2nd bioassay, respectively)

It is noted that this study pre-dates the issue of the formal test method by Mead-Briggs *et al.* (2009) for the extended test design but the methodology used was consistent. This study also followed the standard glass plate test design method (Mead-Briggs *et al.* (2000)). The methods used in this study are consistent with the 2009 version and followed the recommended methods and procedures. The study is therefore considered acceptable.

The LR_{50} and ER_{50} were considered to be >3 x 1.25 L product/ha.

Data Point:	KCP 10.3.2.2/05
Report Author:	[redacted]
Report Year:	2002
Report Title:	Toxicity of JAU 6476 & spiroxamine EC 460 (JAU 6476 & KWG 4168 EC 460) to the cereal aphid parasitoid Aphidius rhopalosiphi (Destefani-Perez) under extended laboratory conditions (aged-residue test)
Report No:	011048030
Document No:	M-056762-01-1
Guideline(s) followed in study:	IOBC proposal (Mead-Briggs & Longley 1997) IOBC (Mead-Briggs et al. 2000) ESCORT recommendation (Barrett et al. 1994)
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and accepted RAR (2010)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

Aphidius rhopalosiphi were exposed to JAU 6476 & Spiroxamine EC 460 under extended laboratory conditions and semi-field aged residues conditions to assess the effects on mortality and fecundity.

The test item was applied twice at a rate of 1.25 L product/ha over 21 days in 200 L deionised water/ha to maize plants. The control was treated with deionised water (200 L/ha) and Dimethoate EC 400 (10 mL product/ha in 200 L/ha of water) was used as a toxic reference treatment.

Exposure to JAU 6476 & Spiroxamine EC 460 did not cause any significant impacts on mortality and fecundity when compared to the control treatment group.

The LR_{50} and ER_{50} were considered to be > 2 x 1.25 L product/ha

I. Materials and Methods

Materials

Test Material JAU 6476 & Spiroxamine EC 460

Lot/Batch #: 06920/0045(0019)

Purity: JAU 6476: 160.4 g/L
Spiroxamine EC 460: 296.2 g/L

Description: Clear dark-yellow liquid

Stability of test compound: Not reported

Reanalysis/Expiry date: 2 November 2001

Density: 0.984 g/cm³

Treatments

Test rates: 2 x 1.25 L product/ha (at an interval of 21 days)

Analysis of test concentrations: Application rates varied within 104 - 109% of nominal rates.

Test organisms

Species:	Parasitic wasp, *Aphidius rhopalosiphi*, adults
Source:	PK Nutzlingszuchten, Industriestraße 38, D-73642 Welzheim
Acclimatisation period:	None
Feeding:	Mortality test: aqueous fructose solution (25% w/v)
Treatment for disease:	None reported

Test design

Test vessel:	Acrylic cylinder (11 cm diameter, 20 cm high) containing 20 wheat seedlings (8 days old) infested with ~100 aphids nymphs (second to third instar) and covered at the top of the cylinder with gauze
Replication:	Mortality test: 4 (1 for reference test) Parasitisation test: 14
No. animals/vessel:	Mortality test: 5 Parasitisation test: 1
Duration of test:	Mortality test: 48 hours Parasitisation test: 12 days

Environmental test conditions

Temperature:	20 - 22°C
Relative humidity:	67 - 73%
Photoperiod:	16 hour light : 8 hour darkness Mortality phase: 2000 lux Parasitisation phase: 4100 lux

Study Design

The parasitic wasp, *Aphidius rhopalosiphi* was exposed to JAU 6476 & Spiroxamine EC 460 under extended laboratory conditions after residual contact exposure under semi-field conditions over 48 hours.

Pupae of the wasps were placed in glass bottles for hatching. Wasps had undergone metamorphosis <48 hours prior to being used in the test. Adult wasps were exposed in four replicates of five wasps per treatment group to the residues of the test item, reference item and control.

The wasps were not fed but only watered for 12 to 18 hours prior to exposure and during the assessments, the wasps were fed with aqueous fructose solution (25% w/v).

Prior to spraying, potted maize plants of each treatment were set up in a 25 m^2 application plot. Then the test items were applied to the plants at a rate of 1.25 L product/ha in 200 L deionised water/ha and again after an interval of 21 days. The control was treated with deionised water (200 L/ha) and Dimethoate EC 400 (10 ml product/ha in 200 L/ha of water) was used as a toxic reference treatment. The test solution was sprayed onto the surface of the plants using a plot-sprayer. Actual applied rates varied from 104 to 109% of target spray volume.

Following the air-drying of the second spray deposit (about 1 hour), acrylic cylinders were set up on the pots with the treated plants. The test units were closed with fine gauze and then placed in a climatic test room. The temperature was 20 to 22°C and was continuously measured.

The first test was conducted after the second spray deposit of the test had dried. Five female wasps were confined to each cage and at 1, 2, 24 and 48 hours after exposure, the number of surviving wasps and their condition were determined. Distinction was drawn between alive, affected, moribund and dead animals. Thirty minutes and two hours after exposure, the behaviour of the wasps were recorded at 3-minute intervals as either on the plant, on the sand, or on the cylinder over a 30-minute period.

After 48 hours, to determine the parasitisation capacity, 14 of the surviving females of the control group and the treated groups were randomly selected and individually confined in acrylic cylinders containing untreated potted wheat plants infested with more than 100 nymphal aphids. The wasps were removed 24 hours later and the parasitisation cages were stored in the climatic room for further 11 days. The number of parasitised aphids was recorded and the parasitisation rate per wasp was determined.

Seven days after the second spray deposit was applied to the maize plants, the process was repeated with further replicates of wasps. The climatic conditions and observation methods were identical to those employed in the first test.

The reference item was applied to the maize plants on day 0 and day 7, at the start of the first and second test.

II. Results and Discussion

Validity criteria according to the study report were met.

- Control mortality to not exceed 12.5% (actual: 0 and 0% in the 1st and 2nd bioassay, respectively)
- Reference item mortality to be at least 50% (actual: 100 and 100% in the 1st and 2nd bioassay, respectively)
- Control reproduction rate to be ≥5 mummies per female (actual: 12.2 and 12.1% in the 1st and 2nd bioassay, respectively)
- No more than two females should produce no mummies (actual: 0 and 0 females producing no mummies in the 1st and 2nd bioassay, respectively)

First test – 0 days after test item applied to plants

No mortality was observed in the test item and control groups. There was no statistically significant difference in reproduction in the test item group when compared to the control.

Table CP 10.3.2.2/05-1 Mortality and fecundity of *Aphidius rhopalosiphi* after 48 hours exposure to test item

Test item concentration (L/ha)	Mortality (%)	Mean no. mummies/female	Fecundity relative to control (%)
Control	0	12.2	-
2 x 1.25	0	11.8	97
Reference	100	-	-

The behaviour assessments in the test item group showed statistically significant differences compared to the control group after exposure to the test item.

Second test – 7 days after test item applied to plants

No mortality was observed in the test item and control groups. There was no statistically significant difference in reproduction in the test item group when compared to the control.

Table CP 10.3.2.2/05-2 Mortality and fecundity of Aphidius rhopalosiphi after 48 hours exposure to test item

Test item concentration (L/ha)	Mortality (%)	Mean no. mummies/female	Fecundity relative to control (%)
Control	0	12.1	
2 x 1.25	0	11.0	91
Reference	100	-	-

The behaviour assessments in the test item group showed that there was no statistically significant differences compared to the control group after exposure to the test item.

III. Conclusion

The parasitic wasp *Aphidius rhopalosiphi* was exposed to JAU 6476 & Spiroxamine EC 460 applied twice to maize plants at a rate of 1.25 L test item/ha in order to assess the effects of exposure on mortality and fecundity.

Exposure to JAU 6476 & Spiroxamine EC 460 did not cause any significant impacts on mortality and fecundity when compared to the control treatment group.

The LR_{50} and ER_{50} were considered to be >2 x 1.25 L product/ha.

Assessment and conclusion by applicant:

The study has been assessed against the validity criteria according to the current extended *Aphidius* test method guideline by Mead-Briggs *et al.* (2009). The validity criteria were met.

- Control mortality to not exceed 10% (actual: 0 and 0% in the 1st and 2nd bioassay, respectively)
- Reference item mortality to be at least 50% (actual: 100 and 100% in the 1st and 2nd bioassay, respectively)
- Control reproduction rate to be ≥5 mummies per female (actual: 12.2 and 12.1% in the 1st and 2nd bioassay, respectively)
- No more than two females should produce no mummies (actual: 0 and 0 females producing no mummies in the 1st and 2nd bioassay, respectively)

It is noted that this study pre-dates the issue of the formal test method by Mead-Briggs *et al.* (2009) for the extended test design but the methodology used was consistent. This study also followed the standard glass plate test design method (Mead-Briggs *et al.* (2000)). The methods used in this study are consistent with the 2009 version and followed the recommended methods and procedures. The study is therefore considered acceptable.

The LR_{50} and ER_{50} were considered to be >2 x 1.25 L product/ha.

Data Point:	KCP 10.3.2.2/06
Report Author:	[redacted]
Report Year:	2005
Report Title:	Effects of prothioconazole & spiroxamine EC 160 + 300 on the ladybird beetle Coccinella septempunctata, extended laboratory study - dose response test
Report No:	26222012
Document No:	M-259116-01-1
Guideline(s) followed in study:	Schmuck et al. 2000; this guideline was modified for exposure of C. septempunctata on natural substrate.
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and accepted RAR (2010)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The purpose of this study was to assess the effects on mortality and reproduction seen during larval and pupal development when exposed to Prothioconazole & Spiroxamine EC 160 + 300.

Under extended laboratory conditions, the LR_{50} was estimated to be higher than 2875 mL product/ha of Prothioconazole & Spiroxamine EC 160 + 300.

Reproduction was >2 fertile eggs per viable female per day at dose rates 12, 58, 266, 1250 and 2875 mL product/ha of Prothioconazole & Spiroxamine EC 160 + 300, so the reproductive output was within the historical data base for control beetles and therefore this parameter was considered as not impacted by the treatment (Schmuck *et al.* 2000). Thus, the ER_{50} was considered to be >2875 mL product/ha of Prothioconazole & Spiroxamine EC 160 + 300.

I. Materials and Methods

Materials

Test Material Prothioconazole & Spiroxamine EC 160 + 300

Lot/Batch #: 06920/0109(0019)

Active substance content: JAU 6476: 160.23 g/L
KWG 4168: 292.93 g/L

Description: Clear dark brown liquid

Stability of test compound: Test substance is considered stable under test conditions

Reanalysis/Expiry date: 22 December 2005

Density: 0.983 g/mL

Treatments

Test rates: 12, 58, 266, 1250 and 2875 mL product/ha

Solvent/vehicle: Deionised water

Analysis of test concentrations: None

Test organisms

Species:	*Coccinella septempunctata* L
Source:	Katz Biotech AG, An der Birkenpfuhlheide 10, D-15837 Baruth (received as eggs)
Acclimatisation period:	Approximately 4 days under test conditions
Feeding:	Larvae: live aphids (*Acyrthosiphon pisum, Megoura viciae*) *ad libitum*. Adults: live aphids (broad bean plant (*Vicia faba*) infested with aphids, *Acyrthosiphon pisum* and *Megoura viciae*; plants were replaced one time a week by fresh ones and if necessary aphids were added additionally), fine grinded pollen and honey *ad libitum*.
Treatment for disease:	NA

Test design

Test vessel:	Exposure units: Detached bean leaves of diameter 55 mm on a wet cotton wool pad (approximately 55 mm in diameter) in a petri dish (approximately 60 mm in diameter). Post-exposure units: Plastic insect rearing cages (40 x 40 x 40 cm) containing plants infested with aphids 3 folded tissue paper sheets served as egg laying surfaces for the adults.
Test medium:	Deionised water
Replication:	Exposure period: 40 per treatment group Post-exposure: 1 per treatment group
No. of animals/vessel:	Exposure period: 1 per replicate Post-exposure: maximum 28 per replicate (all survivors)
Duration of test:	Pre-imaginal mortality phase: 12 – 19 days Reproductive performance: 2 weeks

Environmental test conditions

Temperature:	23 to 26°C
Relative humidity:	61 to 84%
pH:	NA
Photoperiod:	1300 – 2000 lux (16 hour light : 8 hour dark)

Study Design

The purpose of this study was to produce a concentration response curve for mortality effects seen during larval and pupal development when exposed to Prothioconazole & Spiroxamine EC 160 + 300.

The test was conducted at test concentrations of 12, 58, 266, 1250 and 2875 mL product/ha. The water amount in the study was 200 L/ha (corresponding to 2 mg/cm²). The reference substance concentration was 50 mL/ha Perfekthion.

A single application of the control, test substance and reference item was conducted according to agricultural pesticides. The spraying equipment was calibrated using a glass plate of known surface area was sprayed with deionised water. The weight of the glass plate was determined immediately before and after application and the amount of spray deposit per cm² was calculated as the difference between the weight before and after spraying. The procedure was repeated 5 times since the application rate was

within 200 L/ha ± 10 % without changing the adjustment. The uniformity of the deposit distribution was checked visually.

The test species was the Ladybird beetle (Coleoptera, Coccinellidae) *Coccinella septempunctata* L. were obtained from Katz Biotech AG, An der Birkenpfuhlheide 10, D-15837 Baruth. They were obtained as eggs, the larvae age at test start was approximately 4 days old, the 4 day acclimatisation period was under test conditions.

Exposure units were detached leaves (primary leaves were cut from untreated bean plants (*Phaseolus vulgaris*), grown at IBACON - non-GLP -) which were cut to discs with a diameter of approximately 55 mm. Then the discs were treated on their upside with the laboratory spraying equipment and placed each with its treated side upwards on a wet cotton wool pad (approximately 55 mm in diameter) in a petri dish (approximately 60 mm in diameter). The petri dish had a hole for a wick. A Fluon treated cylinder (30 mm high and 40 mm in diameter, approximately the lower 3 mm were not treated with Fluon to avoid contamination of the larvae) was fixed on each leaf by two elastic bands to guarantee a close position on the leaves. Escaping of the larvae and the aphids was prevented by the close position of the cylinder to the leaf and the Fluon on the walls of the cylinder. The exposure units of one treatment group were placed in a bowl. A wick was connected with the cotton wool pad in the exposure units and was wetted. At the end of exposure the cylinders were covered on the top with a plastic lid to prevent emerging beetles from escape.

Post-exposure units for the pre-oviposition and oviposition period were plastic insect rearing cages (40 cm x 40 cm x 40 cm) containing plants infested with aphids. 3 folded tissue paper sheets served as egg laying surfaces for the adults.

For the exposure period 40 replicate test units each housing 1 individual were tested. For the post-exposure period all survivors (maximum 28) were tested.

Individuals were introduced after drying of the test units, 35 to 45 minutes after application. For the exposure period selection of the larvae was impartially performed, transfer by using a fine brush, following the spraying scheme. Only live (alive and apparently unaffected) larvae were introduced. For the post-exposure (pre-oviposition period) only live (alive and apparently unaffected) adults were introduced.

The exposure time was 12 to 19 days, whereas the reference substance exposure was 1 day. The pre-oviposition period was 13 to 15 days. As soon as an adult beetle had appeared it was transferred to an insect rearing cage separated by treatment (except reference item only one day). After 100 % of the viable pupae have hatched in the control and in the test substance groups the beetles were sexed and separated by treatment and transferred back into insect rearing cages. No latecomers occurred. The assessment of the reproductive performance (oviposition period) commenced one week after the control beetles started to lay eggs. At this point the pre-oviposition period was finished.

The oviposition period was 2 weeks. All eggs laid in the subsequent 2 weeks were collected and checked for fertility (larval hatch). The egg laying substrate was replaced daily and that areas on the egg deposition substrate (tissue paper) which contained egg clutches were clipped out. The clipped pieces carrying egg batches were further stored under test conditions until larval hatch. Hatched larvae were removed daily from the egg clutches. If no further hatching of larvae was observed (normally after 4 days), the remaining eggs where no alive larva hatched were determined as infertile.

Food was provided to the larvae as live aphids (*Acyrthosiphon pisum, Megoura viciae*) *ad libitum.* Aphids were replaced or added each day until larvae had entered the pupal stage. The adults were provided live aphids (broad bean plant (*Vicia faba*) infested with aphids, *Acyrthosiphon pisum* and *Megoura viciae*; plants were replaced one time a week by fresh ones and if necessary aphids were added additionally), fine grinded pollen and honey *ad libitum.* water was provided to the adults.

The number of living and dead larvae and pupae and the number of adults hatched was counted daily, except weekends. Mortality of the adults was assessed daily except weekends and the sex of the dead beetles was determined.

The number of eggs was counted daily except weekends within the subsequent two weeks of oviposition. The number of larvae hatched was counted daily. Reproduction was performed where the corrected mortality (M_{corr}) was <60%. No reproduction testing was performed with the reference substance.

Test conditions were recorded with suitable instruments and documented in the raw data. Short-term deviation (<2 hours) from the recommended ranges are partly unavoidable (e.g. due to handling of the set ups) and will normally not result in major disturbances of the test performance and were not reported. The test was conducted in a controlled environment room. The temperature range for the test was 23 to 26°C, relative humidity range from 61 to 84 %, light intensity was 1300 to 2000 lux and the light regime was 16 hour light : 8 hour dark. The size of the test units ensured optimal aeration.

II. Results and Discussion

Validity criteria according to the study report were met:

- The average pre-imaginal mortality of the water treated larvae should not exceed 30% (actual: average = 30%)
- The level of pre-imaginal mortality of the larvae exposed to the reference item should result in a pre-imaginal mortality of at least 40% (actual: average = 100%)
- The number of eggs laid by control females should be ≥2 fertile eggs per viable female per day (actual: 16.6 eggs per viable female per day).

The study is therefore considered acceptable.

Table CP 10.3.2.2/06-1 Effects of Prothioconazole & Spiroxamine EC 160+300 on Larvae and Pupae of *Coccinella septempunctata*

Treatment group	Mortality* [%]	Corrected mortality [%]
Control	30.0	-
12 mL product/ha	45.0	21.4
58 mL product/ha	47.5	25.0
266 mL product/ha	45.0	21.4
1250 mL product/ha	37.5	10.7
2875 mL product/ha	47.5	25.0
Reference item	100.0*	100.0

[Fisher exact test, α = 0.05; n.s. = not significant, * = significant]

[a] the tabulated results represent rounded values calculated on the exact raw data; 40 individuals per treatment group, exposure on treated bean leaves

Table CP 10.3.2.2/06-2 Effects of Prothioconazole & Spiroxamine EC 160 + 300 on the Reproductive Capacity of Adult *Coccinella septempunctata*

Treatment group[a]	Eggs per female[b] per day	Fertile eggs per female per day	Larval hatching rate [%]
Control	21.5 ± 13.8	16.6 ± 10.0	78.1 ± 10.3
12 mL product/ha	15.8 ± 5.8	11.1 ± 4.0	70.9 ± 10.4
58 mL product/ha	12.4 ± 7.7	9.1 ± 6.4	71.7 ± 18.1
266 mL product/ha	63.5 ± 25.4	44.4 ± 15.1	71.6 ± 6.7
1250 mL product/ha	28.9 ± 13.7	19.4 ± 10.1	66.3 ± 16.0

Treatment group [a]	Eggs per female [b] per day	Fertile eggs per female per day	Larval hatching rate [%]
2875 mL product/ha	19.0 ± 9.8	13.9 ± 6.8	74.4 ± 11.5

[Bonferoni-U-Test, α = 0.05; * = significant compared to the control]

note: the tabulated results represent mean and standard deviation calculated on the exact raw data; 1 replicate per treatment group with all survived beetles

[a] adults developed from larvae exposed to spray residues on bean leaves

[b] oviposition started 1 week after the first egg laying was observed in the control and lasted 14 days

III. Conclusion

Under extended laboratory conditions, the LR_{50} was estimated to be higher than 2875 mL product/ha of Prothioconazole & Spiroxamine EC 160 + 300.

Reproduction was >2 fertile eggs per viable female per day at dose rates 12, 58, 266, 1250 and 2875 mL product/ha of Prothioconazole & Spiroxamine EC 160 + 300, so the reproductive output was within the historical data base for control beetles and therefore this parameter was considered as not impacted by the treatment (Schmuck *et al*. 2000). Thus, the ER_{50} was considered to be >2875 mL product/ha of Prothioconazole & Spiroxamine EC 160 + 300.

Assessment and conclusion by applicant:

An assessment of validity was made against the current IOBC test method by Schmuck R *et al*. (2000) which is the guideline to which the study was conducted:

- The average pre-imaginal mortality of the water treated larvae should not exceed 30% (actual: average = 30%)
- The level of pre-imaginal mortality of the larvae exposed to the reference item should result in a pre-imaginal mortality of at least 40% (actual: average = 100%)
- The number of eggs laid by control females should be ≥2 fertile eggs per viable female per day (actual: 16.6 eggs per viable female per day).

The study has been conducted to the current test methodology and the validity criteria have been met. The study is therefore considered acceptable.

The LR_{50} and ER_{50} were considered to be >2.875 L product/ha.

Data Point:	KCP 10.3.2.2/07
Report Author:	[redacted]
Report Year:	2008
Report Title:	Chronic dose-response toxicity (ER50) of Prothioconazole + Spiroxamine EC 160 + 300 g/L to the rove beetle Aleochara bilineata GYLL. under extended laboratory conditions
Report No:	07 10 48 029 A
Document No:	M-298127-01-1
Guideline(s) followed in study:	IOBC Guideline (GRIMM et al. 2000)
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and accepted RAR (2010)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The purpose of this study was to determine a chronic dose-response relationship for reproductive capacity of the rove beetle, *Aleochara bilineata* in an extended laboratory test after contact exposure to spray residues of Prothioconazole & Spiroxamine EC 160 + 300 g/L applied on sandy soil at rates of 175, 340, 661, 1286 and 2500 mL product/ha.

The results of the control group indicated that the test organisms were in good condition and the results of the reference item group indicated that the test system was sensitive to harmful substances.

Statistical analysis of reproduction revealed no significant differences concerning the reproductive capacity between the control and all test item treatment groups.

A calculation of the ER_{50} for reproductive capacity was not possible because the reduction of reproductive capacity was below 50% in all test item treatment groups. The ER_{50} was empirically estimated to exceed the highest tested application rate, *i.e.* >2500 mL product/ha. The LR_{50} was also considered to be >2500 mL product/ha.

I. Materials and Methods

Materials

Test Material: Prothioconazole & Spiroxamine EC 160 + 300 g/L

Lot/Batch #: 2006 009433

Active substance content: JAU 6476: 158.3 g/L and KWG 4168: 292.1 g/L

Description: Clear light brown liquid

Stability of test compound: The test item was diluted under conditions corresponding to those in the field and applied onto sandy soil. The stability under test conditions was therefore of no relevance for this type of experiment and was therefore not reported.

Reanalysis/Expiry date: 14 March 2009

Density: 0.985 g/cm³

Treatments

Test rates:	175, 340, 661, 1286 and 2500 mL product/ha
Solvent/vehicle:	Deionised water
Analysis of test concentrations:	NA

Test organisms

Species:	Adult rove beetle – *Aleochara bilineata* GYLL. (Coleoptera: Staphylinidae)
Source:	In-house culture
Feeding:	Frozen *Chironomus sp.*
Treatment for disease:	Not reported

Test design

Test vessel:	*Test/exposure cage:* plastic vessel (14 cm diameter, 8 cm height) covered with a lid of nylon gauze; filled with wet soil up to a height of about 5 cm (corresponding to approx. 1 kg dry soil moistened with deionised water to approx. 35 % of WHC); side walls above the soil level treated with Fluon® *Hatching/fertility cage:* 2 plastic cages (Bellaplast, 18.3 cm x 13.6 cm x 6.4 cm), one cage with gauze cover (mesh size 2 mm x 2 mm) and a sieve bottom with gauze (mesh size 2 mm x 2 mm), the other cage with intact bottom below for collecting the emerging beetles
Test medium:	LUFA 2.1 (batch Sp 2. 11707)
Replication:	Four replicates per concentration
No. of animals/vessel:	20
Duration of test:	70 days

Environmental test conditions

Temperature:	19 to 22 °C
Relative humidity:	63 to 88%
pH:	NA
Photoperiod:	1000 lux (16 hour light : 8 hour dark)

Study Design

The purpose of this study was to determine a chronic dose-response relationship for reproductive capacity of the rove beetle, *Aleochara bilineata* GYLL. in an extended laboratory test after contact exposure to spray residues of the test item applied on sandy soil (LUFA 2.1).

The test was conducted at test concentrations of 1.5, 175, 340, 661, 1286 and 2500 mL product/ha. The water amount in the study was 400 L/ha. The reference substance concentration was 1.5 L Dimethoate EC 400/L.

The spray fluids were applied once onto the surface of the substrate (moistened soil) without beetles and food, using an automatic application cabin (tracksprayer) to ensure a uniform deposit (400 L/ha = 4

mg/cm², ± 10 %). The quantity of the test solution per area was checked with pre-weighed glass plates placed throughout the application cabin.

The test species was the rove beetle *Aleochara bilineata* GYLL. (Coleoptera: Staphylinidae) which were obtained from a laboratory reared culture. The age of the beetles used in the test was 1 – 7 days. The host organisms were the onion fly, *Delia antique.*

Test/exposure cages were plastic vessel (14 cm diameter, 8 cm height) covered with a lid of nylon gauze, filled with wet soil up to a height of about 5 cm (corresponding to approx. 1 kg dry soil moistened with deionised water to approx. 35 % of WHC); side walls above the soil level treated with Fluon®. Hatching/fertility cages consisted of 2 plastic cages (Bellaplast, 18.3 cm x 13.6 cm x 6.4 cm), one cage with gauze cover (mesh size 2 mm x 2 mm) and a sieve bottom with gauze (mesh size 2 mm x 2 mm), the other cage with intact bottom below for collecting the emerging beetles.

4 replicate test units each housing 20 individuals were tested.

The beetles were added impartially to each replicate of the test cages by placing them on the treated substrate after application, the cages were closed with gauze covers and incubated in a controlled-environment test room (20 ± 2°C, 60 – 90% RH). The beetles were fed approximately one hour after application and then every 2 to 3 days depending on the food consumption. Food (thawed *Chironomid* larvae) was placed on the surface of the substrate.

After 7, 14 and 21 days, approximately 500 onion fly pupae, *Delia antiqua* MEIGEN (Diptera; Anthomyiidae) were added and carefully mixed with the substrate of each test unit so that the pupae were distributed homogeneously within the test unit and completely covered with substrate. The number of pupae was determined by weight on each occasion. Four weeks after test initiation, the exposure phase terminated and the number of beetles alive and dead was recorded. The substrate (containing the sandy and the parasitized onion fly pupae) was allowed to dry for seven days (by removing the test unit lids). After this week, the pupae were removed from the substrate by a sieve (pore size: 2 mm x 2 mm) and placed into hatching cages (one such hatching cage for each exposure cage). The test was terminated, when in the control treatment no more *A. bilineata* adults had emerged from the onion fly pupae.

Four weeks after application the mortality was assessed by counting the number of dead or alive introduced beetles, however, the mortality assessment was considered not necessary for the evaluation of the test and as additional information only. Reproduction was assessed by calculating the average number of hatched beetles of the F_1-generation, five weeks after application and daily, thereafter, up to the final assessment.

The climatic conditions of the test room were recorded continuously for the 70 day test duration. Temperatures ranged from 19 to 22°C and relative humidity ranged from 63 to 88%. Light intensity was 1000 lux and light was provided for 16 hours per day.

II. Results and Discussion

The test accomplished the validity criteria according to GRIMM *et al.* (2000) for conducting the extended laboratory test with *Aleochara bilineata*. This was the test method to which the study followed.

- Average number of hatched beetles of the F_1-generation in the control group should be >400 (actual 568);
- Parasitisation rate in the control should be >26.7% (actual: 37.9%);
- Reduction of the reproductive capacity in the reference item treatment group, relative to control should be ≥50% (actual 99.6%).

Mortality at the end of the exposure period was 3.8, 3.8, 5.0, 6.3, 3.8 and 5.0% in the control, 175, 340, 664, 1286 and 2500 mL product/ha groups, respectively. Mortality in the reference item group was 95%.

By the end of the reproductive phase (day 70) the mean number of hatched beetles per replicate in the control was 568 and the mean number of hatched beetles per host pupa in the control was 0.379.

The mean number of hatched beetles per replicate in the reference group was reduced to 0.3%, compared to the control group.

Table CP 10.3.2.2/07-1 Mortality of *Aleochara bilineata* during the exposure period

Treatment group (mL product/ha)	Number of surviving beetles, which were found 28 days after application	Number of dead beetles	Mortality (%)
Control	77	3	3.8
175	77	3	3.8
340	76	4	5.0
661	75	5	6.3
1286	77	3	3.8
2500	76	4	5.0
Reference item	4	76	95.0

Table CP 10.3.2.2/07-2 Reproduction of *Aleochara bilineata* after exposure

Treatment group (mL product/ha)	Number of hatched beetles of the F_1 generation / introduced female (mean ± s.d.)	Number of hatched beetles / host pupa (mean ± s.d.)	Reproduction of reproductive capacity (relative to the control) R (%)
Control	56.8 ± 2.78	0.379 ± 0.019	-
175	54.9 ± 5.72	0.366 ± 0.038	3.4
340	51.0 ± 2.71	0.340 ± 0.018	10.2
661	54.3 ± 3.68	0.362 ± 0.024	4.4
1286	50.4 ± 5.35	0.336 ± 0.036	11.3
2500	51.6 ± 1.89	0.344 ± 0.013	9.2
Reference item	0.2 ± 0.10	0.002 ± 0.0006	99.6

Table CP 10.3.2.2/07-3 Summarised results

Treatment [mL product/ha]	Reproductive capacity				
	Mean numbers of hatched beetle of the F_1-generation per replicate	Mean number of hatched beetles of the F_1-generation per introduced female (10)	Mean number of hatched beetles/host pupa	Parasitisation rate P (%)	Reduction of reproductive capacity (relative to control) R (%)
Control	568	56.8	0.379	37.9	-
175	549	54.9	0.366	36.6	3.4
340	510	51.0	0.340	34.0	10.2
661	543	54.3	0.362	36.2	4.4

Treatment [mL product/ ha]	Reproductive capacity				
	Mean numbers of hatched beetle of the F_1-generation per replicate	Mean number of hatched beetles of the F_1-generation per introduced female (10)	Mean number of hatched beetles/host pupa	Parasitisation rate P (%)	Reduction of reproductive capacity (relative to control) R (%)
1286	504	50.4	0.336	33.6	11.3
2500	516	51.6	0.344	34.4	9.2

III. Conclusion

The results of the control group indicated that the test organisms were in good condition (average number of hatched beetles of the F_1-generation per replicate: 568). The results of the reference item group indicated that the test system was sensitive to harmful substances (99.6% reduction of reproductive capacity).

Statistical analysis of reproduction revealed no significant differences concerning the reproductive capacity between the control and all test item treatment groups.

A calculation of the ER_{50} for reproductive capacity was not possible because the reduction of reproductive capacity was below 50 % in all test item treatment groups. The ER_{50} was empirically estimated to exceed the highest tested application rate, *i.e.* >2500 mL product/ha. The LR_{50} was also considered to be >2500 mL product/ha.

Assessment and conclusion by applicant:

The study was conducted to the current IOBC test method by Grimm *et al.* (2000) for conducting the extended laboratory test with *Aleochara bilineata*. The validity criteria were met:

- Average number of hatched beetles of the F_1-generation in the control group should be >400 (actual 568);
- Parasitisation rate in the control should be >26.7% (actual: 37.9%);
- Reduction of the reproductive capacity in the reference item treatment group, relative to control should be >50% (actual 99.6%).

The study is therefore considered acceptable.

The LR_{50} and ER_{50} were considered to be > 2500 mL product/ha.

CP 10.3.2.3 Semi-field studies with non-target arthropods

There are no semi-field data available with Prothioconazole & Spiroxamine EC 460 but studies are not considered to be necessary as the available extended laboratory data are sufficient.

CP 10.3.2.4 Field studies with non-target arthropods

There are no field data available with Prothioconazole + Spiroxamine EC 460 but studies are not considered to be necessary as the available extended laboratory data are sufficient.

CP 10.3.2.5 Other routes of exposure for non-target arthropods

Acceptable risks have been demonstrated in the risk assessments for non-target arthropods following application of Prothioconazole + Spiroxamine EC 460 following the proposed use. It is therefore considered that other routes of exposure, *e.g. via* systemic activity, do not need to be specifically investigated. The standard species risk assessments for the in-field and off field exposure *via* contact to

foliar residues were acceptable, therefore additional studies investigating other routes of exposure were not considered necessary.

CP 10.4 Effects on non-target soil meso- and macrofauna

CP 10.4.1 Earthworms

The available earthworm toxicity data for spiroxamine (as Spiroxamine EC 500) and the metabolites of spiroxamine are summarised in the table below.

Table CP 10.4.1-1 Summary of earthworm toxicity studies with spiroxamine and metabolites

Organism	Test item	Test type	Endpoints		Reference
Earthworm (*Eisenia fetida*)	Spiroxamine EC 500 G	56 d Chronic toxicity; 5% peat	**NOEC 158.40 mg/kg soil dw (equivalent to 80 mg a.s./kg soil dw);**	NEW	M-416761-01-1
	Statistical Re-analysis	EC_{10}/EC_{20} not determinable		NEW	M-761531-01-1
Earthworm (*Eisenia fetida*)	KWG 4168-desethyl (M01)	56 d Chronic toxicity; 5% peat	NOEC 100 mg/kg soil dw;	EU	M-281615-01-1
		Statistical Re-analysis	**EC_{10} 93.8 mg/kg soil dw** EC_{20} 120 mg/kg soil dw	NEW	M-760435-01-1
Earthworm (*Eisenia andrei*)	KWG 4168-despropyl (M02)	56 d Chronic toxicity; 10% peat	NOEC 100 mg/kg soil dw; **$NOEC_{Corr}$ 50 mg/kg soil dw[1];** EC_{10} >100 mg/kg soil dw; $EC_{10\,Corr}$ >50 mg/kg soil dw[1]	NEW	M-680755-01-2
Earthworm (*Eisenia fetida*)	KWG 4168-N-oxide (M03)	56 d Chronic toxicity; 5% peat	**NOEC 100 mg/kg soil dw;**	EU	M-281617-01-1
		Statistical Re-analysis	EC_{10} 245 mg/kg soil dw EC_{20} 287 mg/kg soil dw	NEW	M-760434-01-1
Earthworm (*Eisenia fetida*)	KWG 4168-acid (M06)	56 d Chronic toxicity; 10% peat	**NOEC 100 mg/kg soil dw;** EC_{10} >100 mg/kg soil dw;	NEW	M-727123-01-1

EU: previously evaluated as part of the original EU review and listed in EFSA conclusion and DAR
NEW: new study or data generated since the previous EU review or previously not submitted
Values in **bold** have been used in the risk assessment
[1] The NOEC from the study, which was conducted using soil with a 10% peat content, has been divided by 2 to account for lipophilic effects from compounds with a Log Pow>2

The available earthworm toxicity data for prothioconazole and the metabolites of prothioconazole are summarized in the table below.

Table CP 10.4.1-2 Summary of earthworm toxicity studies with prothioconazole, prothioconazole-desthio and prothioconazole-S-methyl

Organism	Test item	Test type	Endpoints		Reference
Earthworm (*Eisenia fetida*)	Prothioconazole	Acute toxicity	LC_{50} >1000 mg a.s./kg soil dw LC_{50corr} >500 mg a.s./kg soil dw [2]	EU	EFSA Conclusion[1]
Earthworm (*Eisenia fetida*)	Prothioconazole EC 250	Acute toxicity	LC_{50} >249.36 mg a.s./kg soil dw LC_{50corr} >125 mg a.s./kg soil dw	EU	
Earthworm (*Eisenia fetida*)	Prothioconazole-desthio	Acute toxicity	LC_{50} >1000 mg/kg soil dw LC_{50corr} >500 mg/kg soil dw [2]	EU	
Earthworm (*Eisenia fetida*)	Prothioconazole-S-methyl	Acute toxicity	LC_{50} >1000 mg/kg soil dw LC_{50corr} >500 mg/kg soil dw [2]	EU	
Earthworm (*Eisenia fetida*)	Prothioconazole EC 250	Chronic toxicity	NOEC 1.33 mg a.s./kg soil dw **$NOEC_{corr}$ 0.665 mg a.s./kg soil dw [2]**	EU	
Earthworm (*Eisenia fetida*)	Prothioconazole-desthio	Chronic toxicity	NOEC 1.0 mg a.s./kg soil dw **$NOEC_{corr}$ 0.5 mg/kg soil dw [2]**	EU	
Earthworm (*Eisenia fetida*)	Prothioconazole-S-methyl	Chronic toxicity	NOEC 100 mg a.s./kg soil dw **$NOEC_{corr}$ 50 mg/kg soil dw [2]**	EU	

Organism	Test item	Test type	Endpoints		Reference
Lumbricius terrestris, L. rubellus, L. castanea, Aporrectodea caliginosa, A. terrestris longa	Prothioconazole EC 250	Field study	3 × 200 g a.s./ha 5 different species identified and assessed. 46% reduction in the number of *A caliginosa* juveniles 7 weeks after first application (2 weeks after final application). No adverse effect 5 month after first application. (Maximum measured soil PEC 0.052 mg prothioconazole/kg based on soil sampling depth of 10 cm which is equivalent to a soil PEC of 0.104 mg prothioconazole/kg over the standard 5 cm depth)	EC	

EU: previously evaluated as part of the original EU review and listed in EFSA conclusion and DAR
[1] EFSA Scientific Report (2007) 106, 1-98. Conclusion on the peer review of Prothioconazole
[2] The NOEC from the study, which was conducted using soil with a 10% peat content, has been divided by 2 to account for lipophilic effects from compounds with a Log Pow ≥ 2
Values in **bold** have been used in the risk assessment

The available earthworm toxicity data for Prothioconazole + Spiroxamine EC 460 are summarized in the table below.

Table CP 10.4.1-3 Summary of earthworm toxicity values using Prothioconazole + Spiroxamine EC 460

Organism	Test item	Test type	Endpoints		Reference
Earthworm (*Eisenia fetida*)	Prothioconazole + Spiroxamine EC 460	56 d Chronic toxicity; 5% peat	**NOEC 32 mg/kg soil dw (equivalent to 9.70 mg SPX/kg soil dw and 5.06 mg PTZ/kg soil dw);**	EU	M-065394-01-1
		Statistical Re-analysis	EC_{10}/EC_{20} not determinable	NEW	M-760884-01-1

EU: previously evaluated as part of the original EU review and listed in EFSA conclusion and DAR
NEW: new study or data generated since the previous EU review or previously not submitted
Values in **bold** have been used in the risk assessment

Toxicity endpoints

In accordance with the Terrestrial Guidance Document (SANCO/10329/2002), to account for the high organic matter content in the test soils, the effect concentrations are corrected by a factor of 2 for lipophilic substances with log P_{ow} > 2. Note that endpoints have only been corrected for studies in which artificial soil with a 10% peat content was used. The Log P_{ow} of spiroxamine is 2.79 and 2.98 at pH 7 for diastomers A and B, respectively and at pH 9 these value are 4.88 and 5.08, respectively. Thus, correction of the endpoint is necessary where artificial soil with a 10% peat content has been used.

The Log P_{ow} of spiroxamine-desethyl (M01) is 2.41, 1.97 and >3.64 at pH 4, 7 and 9, respectively. The Log P_{ow} of spiroxamine-despropyl (M02) is 1.95, 1.41 and >3.44 at pH 4, 7 and 9, respectively. The Log P_{ow} of spiroxamine-N-oxide (M03) is 2.82, 2.59 and 2.63 at pH 4, 7 and 9, respectively. The Log P_{ow} of spiroxamine-carboxylic acid (M06) is 0.45, -0.25 and 0.10 at pH 4, 7 and 9, respectively. Thus, correction of the endpoint for the studies using M01, M02 and M03 would be necessary but only where artificial soil with a 10% peat content has been used.

For the toxicity endpoints of prothioconazole and the associated metabolites the endpoints have been taken directly from the 2007 EFSA Conclusion without any further consideration. Risk assessments for prothioconazole have been presented here but only for completeness and to allow for the risk assessment of this representative formulation, containing spiroxamine, to be conducted. Discussion of the specific endpoints for prothioconazole are not considered to be part of the Renewal of Approval for spiroxamine.

Earthworm reproduction data for spiroxamine technical are not available. However, a valid study is available using Spiroxamine EC 500 which has been submitted here to represent the toxicity of spiroxamine. Note that the reproduction study conducted with the representative formulation, Prothioconazole + Spiroxamine EC 460 is considered to provide the most relevant endpoint for the risk assessment of this representative formulation.

Acute earthworm toxicity data are available for spiroxamine technical, prothioconazole, prothioconazole-desthio and prothioconazole-S-methyl. However, no acute risk assessment has been presented because acute earthworm data are no longer a data requirement under EU Regulations 283/2013 and 284/2013.

Exposure

Full details of the PEC_{soil} calculations have been provided in Document M-CP Section 9 Environmental Fate. The maximum initial and accumulation PEC_{soil} values for spiroxamine and its metabolites, as calculated using FOCUS equations, are given in the table below for the application rate of 375 g a.s./ha.

Table CP 10.4.1-4 PEC_{soil} for spiroxamine and its metabolites

Substance	1 x 375 g a.s./ha		2 x 375 g a.s./ha	
	Max PEC_{soil} (mg/kg)	$PEC_{soil\ accumulation}$ (mg/kg)	Max PEC_{soil} (mg/kg)	$PEC_{soil\ accumulation}$ (mg/kg)
Cereals				
Spiroxamine	0.100	0.035	**0.181**	0.069
M01	0.011	0.015	0.022	**0.030**
M02	0.008	0.011	0.016	**0.021**
M03	0.008	0.010	0.017	**0.022**
M06	0.006	0.013	0.012	**0.025**

PEC_{soil} values used in the risk assessment are highlighted in **bold**

For the risk assessment below the risk envelope approach has been applied in which the PEC_{soil} values for the proposed use with the highest application rate has been used. Thus, the risk assessment has been conducted for the use on cereals at 2 x 1.25 L/ha. For spiroxamine the maximum initial PEC_{soil} value was higher than the accumulation value therefore the maximum initial PEC_{soil} value has been used in

the assessment. However, for all of the spiroxamine metabolites the $PEC_{soil\ accumulation}$ values were greater than the maximum initial PEC_{soil} values therefore the risk assessment for the metabolites has been conducted using the worst case $PEC_{soil\ accumulation}$ values.

For Prothioconazole + Spiroxamine EC 460 the formulation PEC_{soil} was determined to be 0.338 mg/kg soil for the maximum application rate of 1.25 L/ha. Please refer to Document M-CP Section 9 Environmental Fate for further details.

For prothioconazole, prothioconazole-desthio and prothioconazole-S-methyl, PEC_{soil} values of 0.081, 0.075 and 0.021, respectively have been used in the risk assessment. These values have been taken directly from the spiroxamine draft RAR (Spiroxamine dRAR, Volume 3, Annex B.9) and are considered to cover the uses proposed for Prothioconazole + Spiroxamine EC 460.

Isomers

For parent spiroxamine the environmental fate soil degradation data currently suggest that there is no significant selective degradation of isomers over time. As a result, the toxicity data generated using the mixture of the isomers (*i.e.* Spiroxamine EC 500) are considered to represent the toxicity of the isomers in the ratios that would occur in the soil following application. In accordance with the isomer Guidance Document[15] it is therefore not necessary to apply any additional Uncertainty Factor (UF) to the risk assessment (*i.e.* a UF of 1.0 is used).

For the metabolites of spiroxamine there are no chiral data available to be able to make this assessment therefore there is a possibility that selective degradation of isomers could occur in the soil over time. In order to account for any possible increased toxicity to soil organisms as a result of an increase in the ratio of a single isomer, an UF has been applied to the risk assessment of M01, M02, M03 and M06. The UF have been calculated following the recommendations of the isomer Guidance Document and have been presented in the table below.

Table CP 10.4.1-5 Uncertainty Factors determined for the earthworm toxicity data with the metabolites of spiroxamine

Test item	Study reference	Test material batch number	Isomer ratio	UF [1]
Spiroxamine	-	-		1.0 [2]
M01	M-281615-01-1	921103ELB02	A:B 56:42	4.76
M02	M-680755-01-2	AE 1344303-PU-01	A:B 83.1:16.0	12.5
M03	M-281612-01-1	KTS 10324-1-2	D1:D2:D3:D4 27:26:20:27	10.0
M06	M-727423-01-1	AE 1344313-01-03	A:B 47:53	4.26

[1] Changes in stereoisomeric excess are unknown therefore Uncertainty Factor = 100/content of lowest stereoisomer (%) used for ecotox endpoint [as indicted in Table B.1.7 p.30 of isomer GD] and assumes that the toxicological effects of the mixture can be attributed to a single isomer. This assumes that all enantiomer ratios can be safely assumed to be 50:50. For example A:B ratio of 83.1:16 would be 100/(16/2) = UF of 12.5

[2] No additional UF required for parent as no significant change in isomeric ratios has been demonstrated

Risk assessment

The risk assessment has been conducted in accordance with the Terrestrial Guidance Document (SANCO/10329/2002).

[15] Guidance of EFSA on risk assessments for active substances of plant protection products that have stereoisomers as components or impurities and for transformation products of active substances that may have stereoisomers. EFSA Journal 2019;17(8):5804

The effect concentrations for spiroxamine (Spiroxamine EC 500), prothioconazole, Prothioconazole + Spiroxamine EC 460 and for the metabolites are compared to the PEC_{soil} values in the following table.

Table CP 10.4.1-6 Earthworm risk assessment for spiroxamine, prothioconazole, Prothioconazole + Spiroxamine EC 460 and relevant metabolites following application of Prothioconazole + Spiroxamine EC 460 to cereals

Intended use	**Cereals 2 x 1.25 L/ha**			
Chronic effects on earthworms				
Test item	**NOEC/EC_{10}**	**PEC_{soil}**	**UF[1]**	**TER_{LT}[2] (criterion TER ≥ 5)**
Prothioconazole + Spiroxamine EC 460	32 mg product/kg soil	0.328 mg product/kg soil		97.6
	(9.70 mg SPX/kg soil)	0.181 mg a.s./kg soil	1.0	53.6
	(5.06 mg PTZ/kg soil)	0.081 mg a.s./kg soil		62.5
Spiroxamine EC 500	158.4 mg product/kg soil (80 mg a.s./kg soil)	0.181 mg a.s./kg soil	1.0	442
M01	93.8 mg/kg soil	0.030 mg/kg soil	4.76	657
M02	50 mg/kg soil	0.021 mg/kg soil	12.5	190
M03	100 mg/kg soil	0.022 mg/kg soil	10.0	455
M06	100 mg/kg soil	0.025 mg/kg soil	4.26	939
Prothioconazole EC 250	0.665 mg a.s./kg soil	0.081 mg a.s./kg soil	-	8.21
Prothioconazole-desthio	0.5 mg/kg soil	0.075 mg/kg soil	-	6.67
Prothioconazole-S-methyl	50 mg/kg soil	0.021 mg/kg soil	-	2381

[1] Uncertainty Factor applied to account for the unknown effect of a possible change in isomer ratios over time
[2] TER calculated as follows: Toxicity endpoint/(PEC_{soil} × UF)
- Not applicable

The TER_{LT} values for spiroxamine, prothioconazole, Prothioconazole + Spiroxamine EC 460 and the metabolites M01, M02, M03, M06, prothioconazole-desthio and prothioconazole-S-methyl all exceed the trigger value of 5, therefore acceptable risks to earthworms, following the proposed uses of Prothioconazole + Spiroxamine EC 460, can be concluded.

Biodiversity

No relevant scientifically peer-reviewed open literature could be found on spiroxamine or its major metabolites, from an ecotoxicological perspective, on earthworms. Therefore, it is considered that the potential impact of the active substance on biodiversity and the ecosystem, including potential indirect effects *via* alteration of the food web, are covered by the risk assessment for earthworms in this section.

With respect to the earthworm risk assessment, which demonstrated an acceptable outcome with large margins of safety and without the need for risk mitigation, the applicant concludes that the use of the representative lead formulation (Prothioconazole + Spiroxamine EC 460) has a low potential to cause unacceptable effects on biodiversity and the ecosystem *via* trophic interactions. To the best of our knowledge and with the presented safety profile of the spiroxamine a.s., metabolites, and the representative lead formulation, the applicant does not foresee any unacceptable effects on biodiversity and the ecosystem with spiroxamine.

CP 10.4.1.1 Earthworms sub-lethal effects

Data Point:	KCP 10.4.1.1/03
Report Author:	[redacted]
Report Year:	2011
Report Title:	Spiroxamine EC 500 G: Effects on survival, growth and reproduction on the earthworm Eisenia fetida tested in artificial soil
Report No:	KRA-RG-R-120/11
Document No:	M-416761-01-1
Guideline(s) followed in study:	ISO 11268-2: 1998 (E) and OECD 222: April 13, 2004
Deviations from current test guideline:	None
Previous evaluation:	No, not previously submitted -
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The purpose of this study was to assess the effect of Spiroxamine EC 500 G on survival, growth and reproduction on the earthworm *Eisenia fetida*.

In an 8 week study, earthworms were exposed to Spiroxamine EC 500 G at nominal concentrations of 50.00, 89.00, 158.40, 282.00 and 502.00 mg test item/kg dry weight artificial soil. There were 40 earthworms per treatment group, at test initiation they had a mean weight range of 0.25 to 0.47 g.

Exposure to Spiroxamine EC 500 G did not show significant lethal effects to the earthworm *Eisenia fetida* in artificial soil up to the highest test concentration of 502.00 mg test item/kg dry weight soil.

There were no statistically significant differences in growth or reproduction data at test concentrations of up to 158.40 and 282.00 mg test item/kg dry weight soil, respectively. There were statistically significant differences in growth and reproduction data at ≥282.00 and 502.00 mg test item/kg dry weight soil, respectively. The overall NOEC and LOEC values related for growth were therefore determined to be 158.40 and 282.00 mg test item/kg dry weight soil. The overall NOEC and LOEC values related to reproduction were determined to be 282.00 and 502.00 mg test item/kg dry weight soil.

I. Materials and Methods

Materials

Test Material Spiroxamine EC 500 G

Lot/Batch #: EDFL013642

Purity: 508.1 g/L

Description: Yellow-brown liquid

Reanalysis/Expiry date: 8 August 2014

Density: 1.006 g/mL

Treatments

Test rates: Nominal: 50.00, 89.00, 158.40, 282.00 and 502.00 mg/kg soil

Analysis of test concentrations:	No
Test organisms	
Species:	Earthworm, (*Eisenia fetida*)
Source:	[redacted]
Acclimatisation period:	Four days prior to test initiation
Feeding:	Finely ground animal manure
Test design	
Test vessel:	Plastic boxes (16.5 x 12 x 6 cm) covered with perforated plastic lids
Test medium:	Artificial soil: 500 g dry weight
Replication:	Four per treatment group
No. animals/vessel:	Ten animals per test vessel
Duration of test:	Eight weeks
Environmental test conditions	
Temperature:	20 ± 2°C
pH:	5.64 – 6.94
Photoperiod:	16 hours light, 8 hours dark (light intensity: 400 – 800 lux)

Study Design

This study was conducted in order to assess the effect of Spiroxamine EC 500 G on the survival, growth and reproduction on the earthworm *Eisenia fetida* during an exposure into an artificial soil at five different test concentrations.

Ten earthworms were added to each of the four replicate test vessels. Test vessels were plastic boxes (length x width x height *ca.* 16.5 x 12 x 6 cm) with perforated plastic lids.

The test soil consisted of 73.82% industrial quartz sand, 20% kaolinite clay, 5% sphagnum peat, 1% dried cattle manure and 0.18% calcium carbonate. Prepared soil consisted of approximately 500 g of dry weight.

The earthworms were exposed to nominal concentrations of 50.00, 89.00, 158.40, 282.00 and 502.00 mg test item/kg dry weight artificial soil.

Incubation was at 20 ± 2°C with a photoperiod of 16 hours light and 8 hours dark at approximately 400 to 800 lux.

After 4 weeks of exposure, the content of each test vessel was emptied and the adult worms were counted, removed and weighed before the substrate was returned to the respective test units minus the worms. Mortality and behavioural abnormalities (*e.g.* lack of movement and rigidity) were observed at this stage (28 days after application). For the LC_{50} calculation, probit analysis was used with ToxRatPro Version 2.10 statistical software.

At test initiation and after 4 weeks of exposure, the adult test organisms of each vessel were weighed (individually at initiation and together after 4 weeks exposure). Weights were determined by washing the worms and placing them on filter paper to absorb surplus water. The data were statistically evaluated

using Williams multiple sequential t-test. For the EC_{50} calculation, probit analysis was used with ToxRatPro Version 2.10 statistical software.

At test termination, the number of surviving juveniles per test vessel was determined by placing the test vessels in a water bath of 50 to 60 °C and counting all emerging worms. Reproduction was determined by the number of alive juveniles at test termination. The data were statistically evaluated using Welch-t test for inhomogeneous variances with Bonferroni-Holm Adjustment. For the EC_{50} calculation, probit analysis was used with ToxRatPro Version 2.10 statistical software.

The earthworms were fed finely ground cattle manure throughout the test which was added to the soil weekly. At each feeding date, the amount of food consumed by the adult earthworms was visually estimated for each test vessel.

II. Results and Discussion

Validity criteria according to the OECD 222 version of the guideline to which the study was performed were met:

- Each replicate (containing 10 adults) to have produced ≥30 juveniles by the end of the test (actual: 328 to 468)
- The coefficient of variation of reproduction to be ≤30% (actual: 11.6%)
- Adult mortality over the initial 4 weeks of the test to be ≤10% (actual: 0%)

No mortality of adult earthworms were observed after 28 days test duration either in the control group or at any test concentration.

Table CP 10.4.1.1/03-1 Mortality and survival data observed after 28 days exposure

mg test item/kg dry weight artificial soil	Number of surviving worms	Number of dead worms	Mortality (%)
Control Mean S.D.	 10 0	 0 0	 0 0
50.00 Mean S.D.	 10 0	 0 0	 0 0
89.00 Mean S.D.	 10 0	 0 0	 0 0
158.40 Mean S.D.	 10 0	 0 0	 0 0
282.00 Mean S.D.	 10 0	 0 0	 0 0
502.00 Mean S.D.	 10 0	 0 0	 0 0

Statistically significant differences in growth relative to the control were observed at the two highest test concentrations of 282.00 and 502.00 mg test item/kg dry weight soil (results of a Williams multiple sequential t-test, two-sided, α=0.05).

Table CP 10.4.1.1/03-2 Body weight data observed after 28 days exposure

mg test item/kg dry weight artificial soil	Number of surviving worms	Weight of worms (Day 0)	Weight of worms (Day 28)	Weight change (%)
Control				
Mean	10	0.32	0.58	80.87
S.D.	0	0.01	0.03	4.76
50.00				
Mean	10	0.30	0.55	81.02
S.D.	0	0.01	0.02	9.49
89.00				
Mean	10	0.33	0.58	76.84
S.D.	0	0.02	0.01	8.94
158.40				
Mean	10	0.34	0.59	74.07
S.D.	0	0.02	0.03	2.60
282.00				
Mean	10	0.31	0.52	66.05*
S.D.	0	0.01	0.03	8.78
502.00				
Mean	10	0.31	0.48	56.21*
S.D.	0	0.01	0.02	1.80

* Statistically significantly different compared to the control

No statistically significant differences in the number of juveniles were observed at test concentrations of 50.00, 89.00, 158.40 and 282.00 mg test item/kg dry weight soil. Statistically significant differences were observed at the highest test concentration of 502.00 mg test item/kg dry weight soil (results of a Welch-t test for inhomogeneous variances with Bonferroni-Holm adjustment, one-sided smaller, α = 0.05.

Table CP 10.4.1.1/03-3 Juvenile earthworms per test vessel observed after 56 days exposure

mg test item/kg dry weight artificial soil	Mean	S.D.	Coefficient variation	% of control
Control	397.9	46.1	11.6	--
50.00	355.8	38.0	10.7	89.4
89.00	343.3	42.7	12.4	86.3
158.40	373.5	95.0	25.4	93.9
282.00	347.3	40.7	11.7	87.3
502.00	294.3	12.6	4.3	74.0*

* Statistically significantly different compared to the control

A reference item, Derosal (active substance: 36% carbendazim) was tested from 31 January 2011 to 5 April 2011 in a dose response study. Dosages of 0, 1.25, 2.5 and 5.0 mg a.s./kg dry weight soil were tested by application into the artificial soil at test initiation. Mortality of adult earthworms as compared

to control organisms was not observed throughout the test. Observed body weight changes at the application rates of 2.5 and 5.0 mg a.s./kg dry weight soil were statistically significantly reduced in comparison to the control group (α=0.05). Reproduction data at all application rates were statistically significantly reduced in comparison to the control group (α=0.05). The EC_{50} for reproduction was determined to be 1.66 mg a.s./kg dry weight soil with 95% confidence limits between 1.62 - 1.69 mg a.s./kg dry weight soil.

III. Conclusion

Based on the biological and statistical significance of the effects observed on growth and reproduction, it is concluded, that the overall NOEC for this study is 158.40 mg test item/kg dry weight soil (equivalent to 80 mg a.s./kg soil). Thus, the overall LOEC is determined to be 282.00 mg test item/kg dry weight soil (equivalent to 142 mg a.s./kg soil).

Assessment and conclusion by applicant:

The study was conducted to an older version of the current test guideline but the validity criteria remain the same in the current OECD 222 (2016) version. The validity criteria have been met:

- Each replicate (containing 10 adults) to have produced ≥30 juveniles by the end of the test (actual: 328 to 468)
- The coefficient of variation of reproduction to be ≤30% (actual: 11.6%)
- Adult mortality over the initial 4 weeks of the test to be ≤10% (actual: 0%)

The reference substance produced significant effects with an EC_{50} of 1.66 mg a.s./kg soil. This is in line with the values given in the OECD 222 guideline of 1 - 5 mg a.s./kg soil. Thus, the sensitivity of the organisms was confirmed.

The test substance was incorporated into the soil as is now required.

The study is therefore considered to be acceptable.

The NOEC was 158.40 mg test item/kg dry weight soil (equivalent to 80 mg a.s./kg soil).

The results from this study have been statistically re-analysed and a summary of these results is presented below.

Data Point:	KCP 10.4.1.1/04
Report Author:	[redacted]
Report Year:	2020
Report Title:	Calculation of EC10 and EC20 values for Eisenia fetida with spiroxamine EC 500 in a reproduction study
Report No:	0471836-ECO17
Document No:	M-761531-01-1
Guideline(s) followed in study:	None
Deviations from current test guideline:	None
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	not applicable
Acceptability/Reliability:	Yes

Executive Summary

The report M-416761-01-1 on the effects of Spiroxamine EC 500 in the earthworm (*Eisenia fetida*) reproduction study did not provide estimates of EC_{10} or EC_{20} values. Therefore, these values have been

calculated in accordance with the Annex to Com. Reg. 284/2013. Due to the lack of a significant dose response, the determination of EC_{10} and EC_{20} values for reproduction was not possible.

I. Methods

The statistical evaluation was performed with statistical software ToxRat Standard v3.3.0. Due to the lack of a significant dose response between treatment and control, the determination of EC_{10} and EC_{20} values for reproduction was not possible.

II. Results

Due to the lack of a significant dose response, the determination of EC_{10} and EC_{20} values for reproduction was not possible.

III. Conclusion

Due to the lack of a significant dose response between treatment and control, the determination of EC_{10} and EC_{20} values for reproduction was not possible.

Assessment and conclusion by applicant:

The statistical re-evaluation of the reproduction data could not determine reliable EC_{10} and EC_{20} values due to a lack of a dose-response.

The NOEC based on growth of 158.40 mg/kg dws remains the most critical endpoint from this study and has been used in the risk assessment.

The conclusions made in the re-evaluation work are considered to be fully valid.

Data Point:	KCP 10.4.1.1/01
Report Author:	[redacted]
Report Year:	2002
Report Title:	JAU 6476 & spiroxamine EC 460: Effects on reproduction and growth of earthworms Eisenia fetida in artificial soil with 5 percent peat in the test substrate
Report No:	1353/1022
Document No:	M-065394-01-1
Guideline(s) followed in study:	BBA Teil VI, Nr. 2-2 (1994) ISO-Guideline 11268-2 (1998)
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and accepted RAR (2010)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The purpose of this study was to investigate the effects of JAU 6476 & Spiroxamine EC 460 on the reproduction and growth of earthworms in artificial soil with 5% peat in the test substrate.

In an 8-week study, earthworms were exposed to JAU 6476 & Spiroxamine EC 460 at nominal concentrations of 1.0, 3.2, 10, 32 and 100 mg test item/kg dry weight soil. There were 40 earthworms per treatment group; each *ca.* 7 months old and weighing between 300 and 492 mg.

Exposure to JAU 6476 & Spiroxamine EC 460 did not show significant lethal effects to the earthworm *Eisenia fetida* in artificial soil up to the highest test concentration of 100 mg test item/kg soil dry weight.

Statistically significant reductions in earthworm body weight were observed at the test concentration 100 mg test item/kg soil dw. The overall NOEC was therefore determined to be 32 mg test item/kg soil dry weight,

I. Materials and Methods

Materials

Test Material	JAU 6476 & Spiroxamine EC 460
Lot/Batch #:	06920/0045(0019)
Purity:	JAU 6476: 155.00 g/L; KWG 4168: 297.24 g/L
Description:	Dark yellow liquid
Stability of test compound:	In water: test item considered stable under test conditions
Reanalysis/Expiry date:	30 April 2002
Density:	0.981 g/mL
Treatments	
Test rates:	Nominal: 1.0, 3.2, 10, 32 and 100 mg product/kg dry weight soil
Solvent/vehicle:	Deionised water
Test organisms	
Species:	Earthworms (*Eisenia fetida*), *ca.* 7 months old, 300 - 492 mg
Source:	In-house culture
Acclimatisation period:	Four days under test conditions
Feeding:	Finely ground cattle manure
Test design	
Test vessel:	Plastic boxes with perforated transparent lids:18.3 x 13.6 x 6 cm
Test medium:	Artificial soil: 500 g dry weight; added water: 125.8 g
Replication:	Four per treatment group
No. animals/vessel:	Ten animals per test vessel
Duration of test:	Eight weeks
Environmental test conditions	
Temperature:	19 - 21°C
pH:	5.5 – 6.2
Photoperiod:	16 hrs light, 8 hrs dark (light intensity: 475 – 711 lux)

Study Design

This study was conducted in order to assess the effects of JAU 6476 & Spiroxamine EC 460 on the reproduction and growth of earthworms during an exposure into an artificial soil at five different test concentrations.

Adult earthworms approximately 7 months old and weighing between 300 and 492 mg were used in the test. Ten worms were added to each of the four replicate test vessels for each test concentration. Test vessels were plastic boxes of 18.3 x 13.6 x 6 cm with perforated transparent lids.

The test soil consisted of 5% sphagnum-peat, 20% kaolin clay, 0.2% chalk and 74.8% fine quartz-sand. Each vessel was filled with 630.8 g of prepared soil. Prepared soil consisted of approximately 500 g of dry weight artificial soil, approximately 125.8 g of water and 5 g of food.

The earthworms were exposed to nominal concentrations of 1.0, 3.2, 10, 32 and 100 mg product/kg dry weight artificial soil.

Incubation was at 19 to 20°C during acclimatisation and 19 to 21°C during exposure with a photoperiod of 16 hours light and 8 hours dark at approximately 475 to 711 lux.

After 4 weeks of exposure, the content of each test vessel was emptied and the adult worms were counted, removed and weighed before the substrate was returned to the respective test units minus the worms. Mortality and behavioural abnormalities (e.g. lack of movement and rigidity) were observed at this stage (28 days after application). Body weights were determined individually at test initiation and then calculated as a mean per test vessel 28 days after exposure.

At test termination, the number of surviving juveniles per test vessel was determined by placing the test vessels in a water bath and counting all emerging worms. Following this, each vessel was emptied onto a tray and checked by hand for remaining worms. Reproduction was determined by the number of alive juveniles at test termination.

The earthworms were fed finely ground cattle manure throughout the test which was added to the soil weekly. At each feeding date, the amount of food consumed by the adult earthworms was visually estimated for each test vessel.

Mortality data were analysed for significance by using Fisher-exact-test and changes in body weight and reproduction were tested for normal distribution and homogeneity of variance using Kolmogoroff-Smimov-test and Cochran-test. Data of body weight changes and reproduction were normally distributed and homogenous, therefore Dunnett-test was used.

II. Results and Discussion

Validity criteria were assessed in the study report in accordance with the ISO guideline to which the study was conducted.

- Each control replicate to have produced ≥30 juveniles by the end of the test (actual: mean of four replicates was 335)
- The coefficient of variation of reproduction to be ≤30% (actual: 10.1%)
- Adult mortality in control to be ≤10% (actual: 0%)
- Mean loss of biomass in the control to not exceed 20% (actual: biomass increased by 54.5%)

No mortality of adult earthworms were observed after 28 days test duration in the control group and at the test concentrations of 1.0, 3.2, 32 and 100 mg test item/kg soil dry weight. In the test concentration of 10.0 mg test item/kg soil dry weight a slight mortality (2.5%) was observed, which was not significantly different compared to the control (Fisher exact test, α=0.05).

The reproduction rates were not significantly different compared to the control in any test item concentration (Dunnett-test, α=0.05).

Table CP 10.4.1.1/01-1 Survival and reproduction of adult earthworms after 4 and 8 weeks exposure, respectively

Treatment group (mg/kg)	Total surviving adults		Mean adult mortality (4 weeks) (%) ±SD[1]	Mean young per container (8 weeks) ±SD
	Start	4 weeks		
Control	40	40	0.0 ± 0.0	337 ± 34
1.0	40	40	0.0 ± 0.0	375 ± 50
3.2	40	40	0.0 ± 0.0	303 ± 39
10.0	40	39	2.5 ± 5.0	319 ± 39
32	40	40	0.0 ± 0.0	322 ± 12
100	40	40	0.0 ± 0.0	287 ± 49

-=not relevant

[1] mean ± SD = mean ± standard deviation from 4 replicates

The body weight changes of the adult worms after 4 weeks of exposure to JAU 6476 & Spiroxamine EC 460 were not significantly different compared to the control up to and including the test concentration of 32 mg test item/kg soil dry weight, however showed a statistically significant change in the concentration of 100 mg product/kg soil dry weight (Dunnett test, α=0.05).

Table CP 10.4.1.1/01-2 Body weight changes of the adult earthworms

Treatment group (mg/kg)	Mean body weights per earthworm (mg/worm)		Body weight differences (mg/worm)	
	Start	4 weeks	mean ± SD[1]	%[2]
Control	351.9	544.2	192.3 ± 22	54.5 ± 4.6
1.0	353.8	530.4	176.6 ± 12	50.1 ± 4.3
3.2	351.5	542.3	190.8 ± 24	54.2 ± 5.2
10.0	352.0	533.4	181.5 ± 18	51.6 ± 5.4
32	351.0	535.5	184.5 ± 9	52.6 ± 1.5
100	351.7	511.2	159.6 ± 19	45.3* ± 4.8

*=significantly different compared to the control, Dunnett test, α = 0.05

[1] mean ± SD = mean ± standard deviation from 4 replicates

A reference item, Derosal SC 360 (active substance carbendazim) was tested at least once a year in a dose response study. The most recent toxic standard test showed statistically significant effects on reproduction at a concentration of 1.28 mg a.s./kg soil dry weight. The EC_{50} for reproduction was calculated as 1.46 mg a.s./kg soil dry weight.

III. Conclusion

Exposure to JAU 6476 & Spiroxamine EC 460 did not show significant lethal effects to the earthworm *Eisenia fetida* in artificial soil up to the highest test concentration of 100 mg test item/kg soil dry weight.

Statistically significant reductions in earthworm body weight were observed at the test concentration 100 mg test item/kg soil dry weight. The overall NOEC was therefore determined to be 32 mg product/kg soil dry weight, corresponding to 9.70 mg a.s.(spiroxamine)/kg soil or 5.06 mg a.s.(prothioconazole)/kg soil.

Assessment and conclusion by applicant:

The study was not conducted to OECD 222 guidelines but the methodology is the same. Validity criteria according to the OECD 222 (2016) Guideline "Earthworm Reproduction Test (*Eisenia fetida/Eisenia andrei*)" were met

- Each replicate (containing 10 adults) to have produced ≥30 juveniles by the end of the test (actual: mean of four replicates was 337)
- The coefficient of variation of reproduction to be ≤30% (actual: 10.1%)
- Adult mortality over the initial 4 weeks of the test to be ≤10% (actual: 0%)

The reference substance produced significant effects with an EC_{50} of 1.46 mg a.s./kg soil. This is in line with the values given in the OECD 222 guideline of 1 - 5 mg a.s./kg soil. Thus, the sensitivity of the organisms was confirmed.

The test substance was incorporated into the soil as is now required.

The study is therefore considered to be acceptable.

The overall NOEC was therefore determined to be 32 mg product/kg soil dry weight, corresponding to 9.70 mg a.s.(spiroxamine)/kg soil or 5.06 mg a.s.(prothioconazole)/kg soil.

The results from this study have been statistically re-analysed and a summary of these results is presented below.

Data Point:	KCP 10.4.1.1/05
Report Author:	[redacted]
Report Year:	2020
Report Title:	Calculation of EC10 and EC20 values for Eisenia fetida with JAU 6476 & spiroxamine EC 460 in a reproduction study
Report No:	0474836-ECO10
Document No:	M-760884-01-1
Guideline(s) followed in study:	None
Deviations from current test guideline:	None
Previous evaluation:	No, not previously submitted -
GLP/Officially recognised testing facilities:	not applicable
Acceptability/Reliability:	Yes

Executive Summary

The report M-065394-01-1 on the effects of JAU 6467 & Spiroxamine EC 460 in the earthworm (*Eisenia fetida*) reproduction study did not provide estimates of EC_{10} or EC_{20} values. Therefore, these values have been calculated in accordance with the Annex to Com. Reg. 284/2013. Due to the lack of a significant dose response, it is not possible to determine EC_{10} or EC_{20} values for reproduction.

I. Methods

The statistical evaluation was performed with statistical software ToxRat Standard v3.3.0. Due to the lack of a significant dose response, it was not possible to determine EC_{10} or EC_{20} values for reproduction.

II. Results and Discussion

Due to the lack of a significant dose response, it is not possible to determine EC_{10} or EC_{20} values for reproduction.

III. Conclusion

Due to the lack of a significant dose response, it is not possible to determine EC_{10} or EC_{20} values for reproduction.

Assessment and conclusion by applicant:

The statistical re-evaluation of the reproduction data could not determine reliable EC_{10} and EC_{20} values.

The NOEC of 32 mg/kg dws based on growth remains the critical endpoint from this study and shall be used in the risk assessment.

The conclusions made in the re-evaluation work are considered to be fully valid.

Acute earthworm studies

Acute studies using earthworms are no longer a data requirement but for completeness the available acute data with Prothioconazole & Spiroxamine EC 460 have been presented below as supporting information only.

Data Point:	KCP 10.4.1.1/02
Report Author:	[redacted]
Report Year:	2002
Report Title:	Acute toxicity of JAU 6476 & spiroxamine EC 460 to earthworms (Eisenia fetida)
Report No:	LKC/RG 398/02
Document No:	M-063279-01-1
Guideline(s) followed in study:	OECD no. 207 (1984)
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and accepted RAR (2010)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Supportive only

Executive Summary

In a 14-day toxicity study, earthworms were exposed to JAU 6476 & Spiroxamine EC 460 at nominal concentrations of 100, 316 and 1000 mg test substance/kg dry weight soil.

There was no observed mortality in the highest test concentration, therefore, the LC_{50} (at day 14) was >1000 mg test item/kg soil dry weight. There were no significant symptoms or weight alterations at the highest tested concentration of 1000 mg test item/kg soil. The NOEC and LOEC values were ≥1000 and >1000 mg test item/kg soil dry weight, respectively.

I. Materials and Methods

Materials

Test Material	JAU 6476 & Spiroxamine EC 460
Lot/Batch #:	06920/0045(0019)
Purity:	155.00 g/L (JAU 6476); 297.24 g/L (Spiroxamine EC 460)
Description:	Clear dark yellow liquid

Stability of test compound:	Not reported
Reanalysis/Expiry date:	30 April 2002
Density:	Not reported
Treatments	
Test rates:	Nominal: 100, 316 and 1000 mg test item/kg soil dry weight
Solvent/vehicle:	Not reported
Analysis of test concentrations:	No
Test organisms	
Species:	Earthworms (*Eisenia fetida*), adult (>2 months old), average weight: 0.32 g
Source:	Prof Graff, 38104 Braunschweig, Germany
Acclimatisation period:	One day prior to test initiation
Test design	
Test vessel:	1.5 L preserving jars with glass lids
Test medium:	Artificial soil
Replication:	Four replicates
No. animals/vessel:	10 animals per test vessel
Duration of test:	14 days
Environmental test conditions	
Temperature:	20 ± 1°C, 70 – 90% relative humidity
Relative humidity:	70 - 90%
pH:	5.61 - 5.78
Photoperiod:	Continuous illumination of 400 – 800 lux

Study Design

This study was conducted in order to assess the acute toxicity of JAU 6476 & Spiroxamine EC 460 to earthworms in a 14 day study.

Adult earthworms (>2 months old) were placed in a randomised procedure in each test container. The animals were exposed to nominal concentrations of JAU 6476 & Spiroxamine EC 460 at 100, 316 and 1000 mg test item/kg soil dry weight.

The test soil consisted of 69% fine quartz sand, 10% dried finely ground peat, 20% kaolin clay and 1% calcium carbonate.

After 7 days, mortality was observed and all the dead animals were removed from the vessels by hand. At test termination, abnormalities, mortality and weight were observed.

The soil pH was determined using an electronic measuring instrument. Statistical analysis was used for the evaluation of weight alterations of the test organisms.

II. Results and Discussion

Validity criteria according to OECD 207 (1984) were met:

- Control mortality to not exceed 10% (actual: 0%)

No earthworms died at any test concentration over the duration of the test.

Table CP 10.4.1.1/02-1 Observations following exposure to JAU 6476 & Spiroxamine EC 460

Concentration (mg test item/kg dry weight soil)	Mortality (%)	Weight alteration of the survivors (%)
Control	0	+13 ± 5
100	0	+17 ± 8
316	0	+13 ± 2
1000	0	+9 ± 1

Table CP 10.4.1.1/02-2 Summary of endpoints after 14-day exposure to JAU 6476 & Spiroxamine EC 460

Endpoint	NOEC	LOEC	LC_{50}
mg test item/kg soil dw	≥1000	>1000	>1000

III. Conclusion

There were no mortalities, significant symptoms or weight alterations up to the highest test concentration of 1000 mg test item/kg soil dry weight, corresponding with 297.24 mg spiroxamine/kg soil.

The LC_{50} was estimated to be >1000 mg test item/kg soil dry weight.

Assessment and conclusion by applicant:

The test was conducted in accordance with the OECD Guideline No. 207: OECD Guideline for Testing of Chemicals, 'Earthworm, Acute Toxicity Tests' (4 April 1984) which is still the current version. The following validity criterion applies:

- The mortality in the controls should not exceed 10% (actual: 0%)

The study is therefore considered acceptable on the basis that the validity criterion has been met. It is noted that acute earthworm studies are no longer a data requirement therefore this study has been submitted for completeness but considered to be supporting information only.

The LC_{50} was estimated to be >1000 mg test item/kg soil dry weight.

CP 10.4.1.2 Earthworms field studies

No data are available. Field data with Prothioconazole & Spiroxamine EC 460 are not considered necessary as an acceptable risk following the proposed uses has been demonstrated using the available laboratory data.

CP 10.4.2 Effects on non-target soil meso- and macrofauna (other than earthworms)

The available soil meso- and macro-fauna (other than earthworms) toxicity data for spiroxamine and its metabolites are summarised in the table below.

Table CP 10.4.2-1 Summary of soil macro-organism (other than earthworm) toxicity studies with spiroxamine and metabolites

Organism	Test item	Test type	Endpoints		Reference
Folsomia candida	Spiroxamine	28 d Chronic toxicity; 5% peat	**NOEC 32 mg a.s./kg soil dw**	EU	M-289274-01-1
		Statistical Re-analysis	EC_{10}/EC_{20} not determinable	NEW	M-760433-01-1
Folsomia candida	Spiroxamine	28 d Chronic toxicity; 5% peat	NOEC 75 mg a.s./kg soil dw	NEW	M-405276-01-1
		Statistical Re-analysis	EC_{10} 175 mg a.s./kg soil dw EC_{20} 258 mg a.s./kg soil dw	NEW	M-761559-01-1
Folsomia candida	KWG 4168-desethyl (M01)	28 d Chronic toxicity; 5% peat	**NOEC 316 mg/kg soil dw**	EU	M-289320-01-1
		Statistical Re-analysis	EC_{10}/EC_{20} not determinable	NEW	M-760431-01-1
Folsomia candida	KWG 4168-despropyl (M02)	28 d Chronic toxicity; 5% peat	NOEC 316 mg/kg soil dw	EU	M-288905-01-1
		Statistical Re-analysis	**EC_{10} 308 mg/kg soil dw** EC_{20} 402 mg/kg soil dw	NEW	M-760410-01-1
Folsomia candida	KWG 4168-N-oxide (M03)	28 d Chronic toxicity; 5% peat	**NOEC 100 mg/kg soil dw** EC_{10} >100 mg/kg soil dw	NEW	M-687854-01-1
Folsomia candida	KWG 4168-acid (M06)	28 d Chronic toxicity; 5% peat	**NOEC 1000 mg/kg soil dw** EC_{10} >1000 mg/kg soil dw	NEW	M-727126-01-1
Hypoaspis aculeifer	Spiroxamine EC 500	14 d Chronic toxicity; 5% peat	**NOEC 200 mg/kg soil dw (equivalent to 100 mg a.s./kg soil dw)** EC_{10} >200 mg/kg soil dw (equivalent to >100 mg a.s./kg soil dw)	NEW	M-688129-01-1
Hypoaspis aculeifer	Spiroxamine EC 500	14 d Chronic toxicity; 5% peat	NOEC 1000 mg/kg soil dw (equivalent to 505 mg a.s./kg soil dw)	NEW	M-443019-01-1

Organism	Test item	Test type	Endpoints		Reference
Hypoaspis aculeifer	KWG 4168-desethyl (M01)	14 d Chronic toxicity; 5% peat	**NOEC 50 mg/kg soil dw** EC_{10} 94.1 mg/kg soil dw	NEW	M-680684-01-1
Hypoaspis aculeifer	KWG 4168-despropyl (M02)	14 d Chronic toxicity; 5% peat	**NOEC 100 mg/kg soil dw** EC_{10} >100 mg/kg soil dw	NEW	M-680694-01-1
Hypoaspis aculeifer	KWG 4168-N-oxide (M03)	14 d Chronic toxicity; 5% peat	**NOEC 100 mg/kg soil dw** EC_{10} >100 mg/kg soil dw	NEW	M-680687-01-1
Hypoaspis aculeifer	KWG 4168-acid (M06)	14 d Chronic toxicity; 5% peat	**NOEC 1000 mg/kg soil dw** EC_{10} >1000 mg/kg soil dw	NEW	M-727128-02-1

EU: previously evaluated as part of the original EU review and listed in EFSA conclusion and DAR
NEW: new study or data generated since the previous EU review or previously not submitted
Values in **bold** have been used in the risk assessment

The avaialable soil meso- and macro-fauna (other than earthworms) toxicity data for prothioconazole, prothioconazole-desthio and prothioconazole-S-methyl are summarized in the table below.

Table CP 10.4.2-2 Summary of soil macro-organism (other than earthworm) toxicity studies with prothioconazole, prothioconazole-desthio and prothioconazole-S-methyl

Organism	Test item	Test type	Endpoints		Reference
Folsomia candida	Prothioconazole	Chronic toxicity	NOEC 64 mg a.s./kg soil dw **$NOEC_{corr}$ 32 mg a.s./kg soil dw**[2]	EU	EFSA Conclusion[1]
Folsomia candida	Prothioconazole-desthio	Chronic toxicity	NOEC 62.5 mg/kg soil dw **$NOEC_{corr}$ 31.3 mg/kg soil dw**[2]	EU	
Folsomia candida	Prothioconazole-S-methyl	Chronic toxicity	NOEC 31.6 mg/kg soil dw **$NOEC_{corr}$ 15.8 mg/kg soil dw**[2]	EU	
Hypoaspis aculeifer	Prothioconazole	Chronic toxicity	**NOEC 100 mg a.s./kg soil dw**	EU	

EU: previously evaluated as part of the original EU review and listed in EFSA conclusion and DAR
[1] EFSA Scientific Report (2007) 106, 1-98. Conclusion on the peer review of Prothioconazole
[2] The NOEC from the study, which was conducted using soil with a 10% peat content, has been divided by 2 to account for lipophilic effects from compounds with a Log Pow>2
Values in **bold** have been used in the risk assessment

The available soil meso- and macro-fauna (other than earthworms) toxicity data for Prothioconazole + Spiroxamine EC 460 are summarized in the table below.

Table CP 10.4.2-3 Summary of soil macro-organism (other than earthworm) toxicity studies with Prothioconazole + Spiroxamine EC 460

Organism	Test item	Test type	Endpoints		Reference
Folsomia candida	Prothioconazole + Spiroxamine EC 460	28 d Chronic toxicity; 5% peat	NOEC 10 mg/kg soil dw (equivalent to 2.97 mg SPX/kg soil dw and 1.61 mg PTZ/kg soil dw);	EU	M-298769-01-1
		Statistical Re-analysis	EC_{10}/EC_{20} not determinable	NEW	M-761529-01-1
Folsomia candida	Prothioconazole + Spiroxamine EC 460	28 d Chronic toxicity; 5% peat	NOEC 169 mg/kg soil dw (equivalent to 51.0 mg SPX/kg soil dw and 26.0 mg PTZ/kg soil dw);	NEW	M-368461-01-1
		Statistical Re-analysis	EC_{10}/EC_{20} not determinable	NEW	M-761555-01-1
Folsomia candida	Prothioconazole + Spiroxamine EC 460	28 d Chronic toxicity; 5% peat	**NOEC 125 mg/kg soil dw (equivalent to 37.8 mg SPX/kg soil dw and 19.3 mg PTZ/kg soil dw);**	NEW	M-404668-01-1
		Statistical Re-analysis	EC_{10} 134 mg/kg soil dw EC_{20} 165 mg/kg soil dw	NEW	M-761557-01-1
Hypoaspis aculeifer	Prothioconazole + Spiroxamine EC 460	14 d Chronic toxicity; 5% peat	NOEC 316 mg/kg soil dw **EC_{10} 212 mg/kg soil dw (equivalent to 63.4 mg SPX/kg soil dw and 34.6 mg PTZ/kg soil dw);** EC_{20} 329 mg/kg soil dw	NEW	M-611272-01-1

EU: previously evaluated as part of the original EU review and listed in EFSA conclusion and DAR
NEW: new study or data generated since the previous EU review or previously not submitted
Values in **bold** have been used in the risk assessment

Toxicity endpoints

In accordance with the Terrestrial Guidance Document (SANCO/10329/2002), to account for the high organic matter content in the test soils, the effect concentrations would be corrected by a factor of 2 for lipophilic substances with log P_{ow} > 2. The Log P_{ow} of spiroxamine is 2.79 and 2.98 at pH 7 for diastomers A and B, respectively and at pH 9 these values are 4.88 and 5.08, respectively. Thus, correction of the endpoint would be necessary where artificial soil with a 10% peat content has been used. The Log P_{ow} of spiroxamine-desethyl (M01) is 2.41, 1.97 and >3.64 at pH 4, 7 and 9, respectively.

The Log P_{ow} of spiroxamine-despropyl (M02) is 1.95, 1.41 and >3.44 at pH 4, 7 and 9, respectively. The Log P_{ow} of spiroxamine-N-oxide (M03) is 2.82, 2.59 and 2.63 at pH 4, 7 and 9, respectively. The Log P_{ow} of spiroxamine-carboxylic acid (M06) is 0.45, -0.25 and 0.10 at pH 4, 7 and 9, respectively. Thus, correction of the endpoint for the studies using M01, M02 and M03 would also be necessary but only where artificial soil with a 10% peat content has been used. It is noted that all of the available studies using spiroxamine or its metabolites with *Folsomia* and *Hypoaspis* have used artificial soil with a reduced (5%) peat content therefore correction of the endpoint to account for lipophilicity of the substance is not considered to be necessary.

For the toxicity endpoints of prothioconazole and the associated metabolites the endpoints have been taken directly from the 2007 EFSA Conclusion without any further consideration. Risk assessments for prothioconazole have been presented here but only for completeness and to allow for the risk assessment of this representative formulation, containing spiroxamine, to be conducted. Discussion of the specific endpoints for prothioconazole are not considered to be part of the Renewal of Approval for spiroxamine.

For *Hypoaspis aculeifer*, reproduction data for spiroxamine technical are not available. However, data are available using Spiroxamine EC 500 which has been submitted here to represent the toxicity of spiroxamine. Note that the reproduction studies conducted with the representative formulation, Prothioconazole + Spiroxamine EC 460 are considered to provide the most relevant endpoints for the risk assessment of this representative formulation.

Three reproduction studies with *Folsomia candida* are available using Prothioconazole + Spiroxamine EC 460. The first study produced a NOEC of 10 mg product/kg soil dw but this was considered to be low therefore, in order to provide clarification, a second study was conducted which produced a higher NOEC of 169 mg product/kg soil dw. To confirm the NOEC value a third study was conducted which produced a NOEC value of 125 mg product/kg soil dw. It was concluded that the first study had provided an erroneous result and that the latter two studies, which were much more consistent, provided a more realistic result. The lower NOEC out of the two latter studies of 125 mg product/kg soil dw has therefore been used in the risk assessment.

Exposure

Full details of the PEC_{soil} calculations have been provided in Document M-CP Section 9 Environmental Fate. The maximum initial and accumulation PEC_{soil} values for spiroxamine and its metabolites, as calculated using FOCUS equations, are given in the table below for the application rate of 375 g a.s./ha.

Table CP 10.4.2-4 PEC_{soil} for spiroxamine and its metabolites

Substance	1 x 375 g a.s./ha		2 x 375 g a.s./ha	
	Max PEC_{soil} (mg/kg)	$PEC_{soil\ accumulation}$ (mg/kg)	Max PEC_{soil} (mg/kg)	$PEC_{soil\ accumulation}$ (mg/kg)
Cereals				
Spiroxamine	0.100	0.035	**0.181**	0.069
M01	0.011	0.015	0.022	**0.030**
M02	0.008	0.011	0.016	**0.021**
M03	0.008	0.010	0.017	**0.022**
M06	0.006	0.013	0.012	**0.025**

PEC_{soil} values used in the risk assessment are highlighted in **bold**

For the risk assessment below the risk envelope approach has been applied in which the PEC_{soil} values for the proposed use with the highest application rate has been used. Thus, the risk assessment has been conducted for the use on cereals at 2 x 1.25 L/ha. For spiroxamine the maximum initial PEC_{soil} value was higher than the accumulation value therefore the maximum initial PEC_{soil} value has been used in the assessment. However, for all of the spiroxamine metabolites the $PEC_{soil\ accumulation}$ values were greater

than the maximum initial PEC_{soil} values therefore the risk assessment for the metabolites has been conducted using the worst case $PEC_{soil\ accumulation}$ values.

For Prothioconazole + Spiroxamine EC 460 the formulation PEC_{soil} was determined to be 0.328 mg/kg soil for the maximum application rate of 1.25 L/ha. Please refer to Document M-CP Section 9 Environmental Fate for further details.

For prothioconazole, prothioconazole-desthio and prothioconazole-S-methyl, PEC_{soil} values of 0.081, 0.075 and 0.021, respectively have been used in the risk assessment. These values have been taken directly from the spiroxamine draft RAR (Spiroxamine dRAR, Volume 3, Annex B.9) and are considered to cover the uses proposed for Prothioconazole + Spiroxamine EC 460.

Isomers

For parent spiroxamine the environmental fate soil degradation data currently suggest that there is no significant selective degradation of isomers over time. As a result, the toxicity data generated using the mixture of the isomers (*i.e.* spiroxamine or Spiroxamine EC 500) are considered to represent the toxicity of the isomers in the ratios that would occur in the soil following application. In accordance with the isomer Guidance Document[16] it is therefore not necessary to apply any additional Uncertainty Factor (UF) to the risk assessment (*i.e.* a UF of 1.0 is used).

For the metabolites of spiroxamine there are no chiral data available to be able to make this assessment therefore there is a possibility that selective degradation of isomers could occur in the soil over time. In order to account for any possible increased toxicity to soil organisms as a result of an increase in the ratio of a single isomer, an UF has been applied to the risk assessment of M01, M02, M03 and M06. The UF have been calculated following the recommendations of the isomer Guidance Document and have been presented in the table below.

Table CP 10.4.2-5 Uncertainty Factors determined for the soil meso- and macro-fauna toxicity data with the metabolites of spiroxamine

Test item	Study reference	Test material batch number	Isomer ratio	UF [1]
Folsomia candida				
Spiroxamine	-	-	-	1.0 [2]
M01	M-289321-01-1	921103ELB02	A:B 56:42	4.76
M02	M-288905-01-1	921103ELB03	A:B 55:42	4.76
M03	M-687854-01-1	M26999	D1:D2:D3:D4 22:21:26:31	9.52
M06	M-727126-01-1	AE 1344313-01-03	A:B 47:53	4.26
Hypoaspis aculeifer				
Spiroxamine	-	-	-	1.0 [2]
M01	M-680684-01-1	AE 1344302-PU-01	A:B 52:48	4.17
M02	M-680694-01-1	AE 1344303-PU-01	A:B 83.1:16.0	12.5
M03	M-680687-01-1	M26999	D1:D2:D3:D4 22:21:26:31	9.52
M06	M-727128-02-1	AE 1344313-01-03	A:B 47:53	4.26

[16] Guidance of EFSA on risk assessments for active substances of plant protection products that have stereoisomers as components or impurities and for transformation products of active substances that may have stereoisomers. EFSA Journal 2019;17(8):5804

[1] Changes in stereoisomeric excess are unknown therefore Uncertainty Factor = 100/content of lowest stereoisomer (%) used for ecotox endpoint [as indicted in Table B.1, p.30 of isomer GD] and assumes that the toxicological effects of the mixture can be attributed to a single isomer. This assumes that all enantiomer ratios can be safely assumed to be 50:50. For example A:B ratio of 83.1:16 would be 100/(16/2) = UF of 12.5

[2] No additional UF required for parent as no significant change in isomeric ratios has been demonstrated

Risk assessment

The risk assessment has been conducted in accordance with the Terrestrial Guidance Document (SANCO/10329/2002).

The effect concentrations for spiroxamine (Spiroxamine EC 500), prothioconazole, Prothioconazole + Spiroxamine EC 460 and for the metabolites are compared to the PEC_{soil} values in the following table.

Table CP 10.4.2-6 Soil meso- and macro-fauna (other than earthworms) risk assessment for spiroxamine, prothioconazole, Prothioconazole + Spiroxamine EC 460 and relevant metabolites following application of Prothioconazole + Spiroxamine EC 460 to cereals

Intended use	**Cereals 2 x 1.25 L/ha**			
Chronic effects on soil meso- and macro-fauna (other than earthworms)				
Test item	**NOEC/EC_{10}**	**PEC_{soil}**	**UF**	**TER_{LT} [2] (criterion TER ≥ 5)**
Folsomia candida				
Prothioconazole + Spiroxamine EC 460	125 mg product/kg soil	0.328 mg product/kg soil	-	381
	(37.8 mg SPX/kg soil)	0.181 mg a.s./kg soil	1.0	209
	(19.3 mg PTZ/kg soil)	0.081 mg a.s./kg soil	-	238
Spiroxamine	32 mg a.s./kg soil	0.181 mg a.s./kg soil	1.0	177
M01	316 mg/kg soil	0.030 mg/kg soil	4.76	2213
M02	308 mg/kg soil	0.021 mg/kg soil	4.76	3081
M03	100 mg/kg soil	0.022 mg/kg soil	9.52	477
M06	1060 mg/kg soil	0.025 mg/kg soil	4.26	9390
Prothioconazole	32 mg a.s./kg soil	0.081 mg a.s./kg soil	-	395
Prothioconazole-desthio	31.3 mg/kg soil	0.075 mg/kg soil dw	-	417
Prothioconazole-S-methyl	35.8 mg/kg soil	0.021 mg/kg soil dw	-	752
Hypoaspis aculeifer				
Prothioconazole + Spiroxamine EC 460	212 mg product/kg soil	0.328 mg product/kg soil	-	646
	(63.4 mg SPX/kg soil)	0.181 mg a.s./kg soil	1.0	350
	(34.6 mg PTZ/kg soil)	0.081 mg a.s./kg soil	-	427
Spiroxamine EC 500	200 mg product/kg (100 mg a.s./kg soil)	0.181 mg a.s./kg soil	1.0	552
M01	50 mg/kg soil	0.030 mg/kg soil	4.17	400
M02	100 mg/kg soil	0.021 mg/kg soil	12.5	381
M03	100 mg/kg soil	0.022 mg/kg soil	9.52	477

Intended use	Cereals 2 x 1.25 L/ha			
Chronic effects on soil meso- and macro-fauna (other than earthworms)				
Test item	**NOEC/EC_{10}**	**PEC_{soil}**	**UF[1]**	**TER_{LT}[2] (criterion TER ≥ 5)**
M06	1000 mg/kg soil	0.025 mg/kg soil	4.26	9390
Prothioconazole	100 mg a.s./kg soil	0.081 mg a.s./kg soil	-	1235

[1] Uncertainty Factor applied to account for the unknown effect of a possible change in isomer ratios overtime
[2] TER calculated as follows: Toxicity endpoint/(PEC_{soil} × UF)
- Not applicable

The TER_{LT} values for spiroxamine, prothioconazole, Prothioconazole + Spiroxamine EC 460 and the metabolites M01, M02, M03, M06, prothioconazole-desthio and prothioconazole-S-methyl all exceed the trigger value of 5, therefore acceptable risks to soil meso- and macro-fauna (other than earthworms), following the proposed uses of Prothioconazole + Spiroxamine EC 460, can be concluded.

Biodiversity

No relevant scientifically peer-reviewed open literature could be found on spiroxamine or its major metabolites, from an ecotoxicological perspective, on soil meso- and macrofauna (other than earthworms). Therefore, it is considered that the potential impact of the active substance on biodiversity and the ecosystem, including potential indirect effects *via* alteration of the food web, are covered by the risk assessment for soil meso- and macrofauna (other than earthworms) in this section.

With respect to the risk assessment for non-target soil meso- and macrofauna, which demonstrated an acceptable outcome with large margins of safety and without the need for risk mitigation, the applicant concludes that the use of the representative lead formulation (Prothioconazole + Spiroxamine EC 460) has a low potential to cause unacceptable effects on biodiversity and the ecosystem *via* trophic interactions. To the best of our knowledge and with the presented safety profile of the spiroxamine a.s., metabolites and the representative lead formulation, the applicant does not foresee any unacceptable effects on biodiversity and the ecosystem with spiroxamine.

CP 10.4.2.1 Species level testing

Data Point:	KCP 10.4.2.1/01
Report Author:	[redacted]
Report Year:	2008
Report Title:	Prothioconazole + spiroxamine EC 460 (160+300) G: Influence on the reproduction of the Collembola species Folsomia candida tested in artificial soil with 5 percent peat
Report No:	FRM-COLL-57/07
Document No:	M-298769-01-1
Guideline(s) followed in study:	ISO 11267 (1999)
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and accepted RAR (2010)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

Collembola (*Folsomia candida*) aged 10 to 12 days were exposed to Prothioconazole + Spiroxamine EC 460 (160+300) G incorporated into soil in a 4-week study to assess effects on reproduction.

Test organisms were exposed to 10, 20, 40, 80, 160 and 320 mg test item/kg soil dry weight and to a water control. Betosip (a.s.: phenmedipham) was used as a toxic standard in accordance with ISO 11267 (1999) guidelines.

A statistically significant reduction in number of juveniles compared to the control was observed in the treatment groups at 20, 40, 80, 160 and 320 mg test item/kg soil dry weight, resulting in reductions of 30.5, 26.8, 22.8, 42.1 and 85.1 %, respectively.

The NOEC and LOEC for reproduction were 10 and 20 mg test item/kg soil dry weight, respectively.

I. Materials and Methods

Materials

Test Material Prothioconazole + Spiroxamine EC 460 (160+300) G

Lot/Batch #: 2006-009433

Purity: a) Prothioconazole: 158.3 g/L
b) Spiroxamine: 292.1 g/L

Description: Clear, light brown, liquid

Stability of test compound: Not reported

Reanalysis/Expiry date: 14 March 2009

Density: 0.985 g/mL

Treatments

Test rates: 10, 20, 40, 80, 160, 320 mg test item/kg soil dry weight

Solvent/vehicle: Water

Analysis of test concentrations: No

Test organisms

Species:	Collembola, *Folsomia candida*
Source:	Bred at Bayer CropScience. Strain originally obtained from Ibacon, Institute for Analytic and Consulting, GmbH, D- 64380 Rossdorf.
Feeding:	Approximately 2 mg granulated dry yeast at the start of the study and after 14 days.
Treatment for disease:	None reported

Test design

Test vessel:	Glass vessels (volume: 140 mL, diameter: 5 cm) covered with glass lids
Test medium:	Artificial soil according to OECD 207 (1984). With respect to the properties of the test item (log P_{ow} > 2) 5% peat instead of 10% peat was used considering the influence on bioavailability
Replication:	5 (+1 without Collembola for measurement of soil moisture during the test and pH and soil moisture at the end of the study)
No. animals/vessel:	10
Duration of test:	28 days

Environmental test conditions

Temperature:	20 ± 2°C
Water holding capacity:	Test start: 47.66 – 49.75% Test end: 40.47 – 45.84%
Photoperiod:	16 h light : 8 h dark at 608 – 624 lux

Study Design

Collembola (*Folsomia candida*) were exposed to Prothioconazole + Spiroxamine EC 460 (160+300) G over 4 weeks to assess effects on mortality and reproduction. The test concentrations were based on a pre-test (non-GLP) with *Folsomia candida* and the same test item.

The Collembola were 10 to 12 days old at the start of the test. For each replicate, 10 of the juvenile Collembola were placed in the test vessels, which had been prepared with the test item solution mixed with artificial soil. The soil was aligned with OECD 207 (1984) standard, but with 5% peat instead of 10% due to considerations on the influence on bioavailability with respect to the test item. Approximately 30 g (wet weight) of the test substrate was filled in each test vessel, avoiding compression. Water was added until 50% water holding capacity was achieved.

The artificial soil was kept at 18 to 22°C, with the temperature continuously recorded by a thermohydrograph integrated in the climatic chamber. The test vessels were kept under 608 to 624 lux with a photoperiod of 16 h light: 8 h dark, monitored by an integrated luxmeter in the climatic chamber.

Five replicates were exposed to control (water) treatment, and to 10, 20, 40, 80, 160 and 320 mg test item/kg soil dry weight. During the study, the test organisms were fed with granulated dry yeast.

A reference test with the toxic standard, Betosip, was performed at least once a year to ensure that the laboratory test conditions are adequate and to verify that the response of the test organisms does not change significantly over time.

After 14 days, water content was checked and replenished if water loss exceeded 2% of initial content. Food was also checked at this time and the Collembola were fed again if necessary. Mortality and reproduction were reported after 28 days and were determined by the number of dead adult Collembola and the number of living juveniles detected using digital images.

II. Results and Discussion

Validity criteria according to the study report were met:

- Mean adult mortality <20% at the end of the test (actual: 2%);
- The mean number of juveniles per vessel ≥100 at the end of the test (actual: 1464);
- The coefficient of variation calculated for the number of juveniles <30% (actual: 19.9%).

Prothioconazole & Spiroxamine EC 460 (160+300) G did not show lethal effects to the springtail Folsomia candida in artificial soil up to the test concentration of 160 mg test item/kg soil dry weight. At 320 mg test item/kg soil dry weight there was 54% mortality.

Table CP 10.4.2.1/01-1 Survival of adult Collembola after 4 weeks treatment (n=10/replicate)

	Treatment (mg test item/kg soil dry weight)						
	Control	**10**	**20**	**40**	**80**	**160**	**320**
Mean[1]	9.8	9.8	8.0	8.6	8.6	9.8	4.6
SD[1]	0.4	0.4	1.6	2.1	1.5	0.4	1.1
% Mortality[2]	2.0	2.0	20.0	14.0	14.0	2.0	54.0

[1] mean and standard deviation (SD) of five replicates

[2] formula: ((initial placed organisms per vessel – mean of surviving adults per vessel) / 10) * 100

There were statistically significant reductions in the the numbers of juveniles produced at concentrations of ≥20 mg test item/kg soil dry weight. The NOEC for reproduction was therefore set at 10 mg test item/kg soil dry weight.

Table CP 10.4.2.1/01-2 Reproduction of the Collembola after 4 weeks treatment (juveniles/replicate)

	Treatment (mg test item/kg soil dry weight)						
	Control	**10**	**20**	**40**	**80**	**160**	**320**
Mean[1]	1464.0	1429.6	1022.6	1072.2	1130.2	847.8	217.6
SD[1]	291.4	131.2	173.0	182.4	71.5	109.8	69.5
CV[2]	19.9	-	-	-	-	-	-
% of Control[3]	-	97.7	69.8*	73.2*	77.2*	57.9*	14.9*

[1] mean and standard deviation (SD) of five replicates
[2] Coefficient of Variation
[3] formula: mean number of juveniles per treatment group * 100 / mean number of juveniles per control group
- = not applicable
* = significantly different compared to the control (Dunnett's Test, one-sided-smaller, α=0.05)

To demonstrate the sensitivity of the test system Betosip (Phenmedipham 15.4 %) as a toxic standard was tested (once a year) at concentrations of 50, 100 and 200 mg test item/kg artificial soil dry weight. In the most recent test the mortality rate of adult Collembola was 8 %, 16 % and 34 % at 50, 100 and 200 mg Betosip/kg artificial soil dry weight. In the treatment groups 100 and 200 mg Betosip/kg artificial soil dry weight the number of juveniles were statistically significant reduced in comparison to

the control. The NOEC was therefore determined to be 50 mg Betosip (7.7 mg a.s.)/kg soil dry weight. the LOEC was determined to be 100 mg Betosip (15.4 mg a.s.)/kg soil dry weight. The results were considered to demonstrate sufficient sensitivity of the test organism.

III. Conclusion

Collembola (*Folsomia candida*) aged 10 to 12 days were exposed to Prothioconazole + Spiroxamine EC 460 (160+300) G incorporated into soil in a 4-week study to assess effects on reproduction. Statistically significant reductions were measured in the the numbers of juveniles produced at concentrations of ≥20 mg test item/kg soil dry weight. The $NOEC_{reproduction}$ was therefore set at 10 mg test item/kg soil dry weight (equivalent to 2.97 mg spiroxamine/kg dry weight soil and 1.61 mg Prothioconazole/kg dry weight soil, respectively). The $LOEC_{reproduction}$ was reported at 20 mg test item/kg artificial soil dry weight.

Assessment and conclusion by applicant:

This study was previously evaluated and accepted in the dRAR (2017).

The study was conducted to the ISO test guideline therefore validity criteria according to the OECD 232 guideline (2016) have been assessed. The following criteria were met:

- Mean adult mortality <20% at the end of the test (actual: 2%);
- The mean number of juveniles per vessel ≥100 at the end of the test (actual: 1464);
- The coefficient of variation calculated for the number of juveniles <30% (actual: 19.9%).

The study was not conducted specifically to the OECD 232 test guideline but the methods and procedures used are consistent. It is noted that OECD 232 recommends the use of boric acid as a reference substance, but this study used phenmedipham, however, sufficient sensitivity was considered to have been demonstrated therefore the results are considered to be valid.

The study is therefore considered acceptable.

The $NOEC_{reproduction}$ was set at 10 mg test item/kg soil dry weight (equivalent to 2.97 mg spiroxamine/kg dry weight soil and 1.61 mg prothioconazole/kg dry weight soil, respectively).

The results from this study have been statistically re-analysed and a summary of these results is presented below.

Data Point:	KCP 10.4.2.1/02
Report Author:	[redacted]
Report Year:	2020
Report Title:	Calculation of EC10 and EC20 values for Folsomia candida with prothioconazole + spiroxamine EC 460 G in a reproduction study
Report No:	0471836-ECO16
Document No:	M-761529-01-1
Guideline(s) followed in study:	none
Deviations from current test guideline:	None
Previous evaluation:	No, not previously submitted -
GLP/Officially recognised testing facilities:	not applicable
Acceptability/Reliability:	Yes

Executive Summary

The report M-298769-01-1 on the effects of prothioconazole + spiroxamine EC 460 G in a springtail (*Folsomia candida*) reproduction study did not provide estimates of EC_{10} or EC_{20}. Therefore, these values have been calculated in accordance with the Annex to Com. Reg. 284/2013. Due to the lack of a significant dose response, it was not possible to determine EC_{10} or EC_{20} values for reproduction.

I. Methods

The statistical evaluation was performed with statistical software ToxRat Standard v3.3.0. Due to the lack of a significant dose response on the reproduction, when compared to the control, it was not possible to calculate reliable EC_{10} and EC_{20} values.

II. Results

Due to the lack of a significant dose response on the reproduction, when compared to the control, it was not possible to calculate reliable EC_{10} and EC_{20} values.

III. Conclusion

Due to the lack of a significant dose response, it was not possible to determine EC_{10} or EC_{20} values for reproduction.

Assessment and conclusion by applicant:

The statistical re-evaluation of the reproduction data could not determine reliable EC_{10} and EC_{20} values.

The NOEC of 10 mg/kg dws remains the critical endpoint from this study.

The conclusions made in the re-evaluation work are considered to be fully valid.

Data Point:	KCP 10.4.2.1/03
Report Author:	[redacted]
Report Year:	2010
Report Title:	Prothioconazole + spiroxamine EC 460 (160+300) G: Influence on the reproduction of the collembolan species Folsomia candida tested in artificial soil
Report No:	FRM-COLL-89/10
Document No:	M-368461-01-1
Guideline(s) followed in study:	OECD 232 adopted, September 07, 2009: OECD Guidelines for Testing Chemicals - Collembolan Reproduction Test in Soil
Deviations from current test guideline:	None
Previous evaluation:	No, not previously submitted -
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

Collembola species (*Folsomia candida*) aged 10 to 12 days were exposed to Prothioconazole + Spiroxamine EC 460 (160+300) G incorporated into soil in a 4-week study to assess effects on mortality and reproduction.

Test organisms were exposed to 22, 33, 50, 75, 113 and 169 mg test item/kg soil and to a water control. Boric acid was used as a toxic standard in accordance with OECD 232 (2009) guidelines.

There were no statistically significant results observed. The NOEC and LOEC for reproduction were ≥169 and >169 mg test item/kg soil dry weight, respectively.

I. Materials and Methods

Materials

Test Material: Prothioconazole + Spiroxamine EC 460 (160+300) G
(PTZ+SPX EC 460 (160+300) G)

Lot/Batch #: EDFL0001V7

Purity: a) Prothioconazole: 151.1 g/L
b) Spiroxamine: 296.3 g/L

Description: Dark yellow, clear liquid

Stability of test compound: Not reported

Reanalysis/Expiry date: 13 July 2011

Density: 0.981 g/mL

Treatments

Test rates: 22, 33, 50, 75, 113 and 169 mg test item/kg soil dry weight

Solvent/vehicle: Water

Analysis of test concentrations: None

Test organisms

Species:	Springtails, *Folsomia candida* (Collembola, Isotomidae)
Source:	Bred at Bayer CropScience. Strain originally obtained from Ibacon, Institute for Analytic and Consulting, GmbH, D- 64380 Rossdorf.
Feeding:	Approximately 2 mg granulated dry yeast at the start of the study and after 14 days.
Treatment for disease:	None reported

Test design

Test vessel:	Glass vessels (volume: 140 mL, diameter: 5 cm) covered with glass lids
Test medium:	Artificial soil according to OECD 207 (1984). This is in line with OECD 232 (2016). 5% peat content
Replication:	8 replicates for the control group and 4 replicates for each treatment group (+1 replicate for each group without collembolans for measurement of pH and soil moisture at the end of the study)
No. animals/vessel:	10
Duration of test:	28 days

Environmental test conditions

Temperature:	20 ± 2°C
Photoperiod:	16 h light : 8 h dark at 628-634 lux

Study Design

This study was conducted in order to assess the influence on reproduction of Prothioconazole + Spiroxamine EC 460 (160+300) G on Collembola in an inhibition of reproduction test over 4 weeks.

The Collembola (*Folsomia candida*) were 10 to 12 days old at the start of the test. For each replicate, 10 of the juvenile Collembola were placed in the test vessels, which had been prepared with the test item solution mixed with artificial soil. The soil was aligned with OECD 207 (1984) standard. Approximately 30 g (wet weight) of the test substrate was filled in each test vessel, avoiding compression. Water was added to the artificial soil until 50% water holding capacity was achieved.

The artifical soil was kept at 18 to 22°C, with the temperature continuously recorded by a thermo hygrograph integrated in the climatic chamber. The test vessels were also kept at 628 to 634 lux under a photoperiod of 16 h light: 8 h dark, monitored by integrated luxmeter of the climatic chamber.

Eight replicates were exposed to control treatments and four replicates were exposed to treatments of 22, 33, 50, 75, 113 and 169 mg test item/kg soil dry weight. During the study, the test organisms were fed with granulated dry yeast.

The most recent reference test with the toxic standard, boric acid, was performed at concentrations of 44, 67, 100, 150 and 225 mg Boric acid/kg artificial soil dry weight.

After 14 days, water content was checked and replenished if water loss exceeded 2% of initial content. Food was also checked at this time and the Collembola were fed again if necessary.

Mortality and reproduction were reported after 28 days and were determined by the number of dead adult Collembola and the number of living juveniles detected using digital images.

II. Results and Discussion

Validity criteria according to the OECD 232 guideline to which the study was conducted were met:

- Mean adult mortality <20% at the end of the test (actual: 5%);
- The mean number of juveniles per vessel ≥100 at the end of the test (actual: 1317);
- The coefficient of variation calculated for the number of juveniles <30% (actual: 7.6%).

In the control group 5 % of the adult *Folsomia candida* died which is below the allowed maximum of < 20 % mortality. A LC_{50} could not be calculated and is considered to be >169 mg test item/kg soil dry weight.

Table CP 10.4.2.1/03-1 Survival of adult Collembola after 4 weeks treatment (n=10/replicate)

		Treatment (mg test item/kg soil dry weight)						
		Control	22	33	50	75	113	169
Mean[1]		9.5	9.8	9.0	8.5	9.0	8.8	9.3
SD[1]		0.8	0.5	1.4	0.6	0.8	1.5	1.0
% mortality[2]		5.0	2.5	10.0	15.0	10.0	12.5	7.5

1 mean and standard deviation (SD) of eight replicates in control group and 4 in treatment groups

2 formula: ((initial placed organisms per vessel – mean of surviving adults per vessel) / 10) * 100

Concerning the number of juveniles statistical analysis (William's-t test, one-sided smaller, α = 0.05) revealed no significant difference between control and any treatment group. Therefore the No-Observed-Effect-Concentration (NOEC) for reproduction was ≥169 mg test item/kg soil dry weight. The Lowest-Observed-Effect-Concentration (LOEC) for reproduction was >169 mg test item/kg soil dry weight. An EC_{50} could not be calculated and was considered to be > 169 mg test item/kg soil dry weight

Table CP 10.4.2.1/03-2 Reproduction of the Collembola after 4 weeks treatment (juveniles/replicate)

	Treatment (mg test item/kg soil dry weight)						
	Control	22	33	50	75	113	169
Mean[1]	1316.9	1356.0	1231.8	1281.0	1457.0	1260.0	1261.0
SD[1]	100.6	56.8	206.4	116.1	190.7	160.4	211.2
CV[2]	7.6	-	-	-	-	-	-
% of Control[3]	-	103.0	93.5	97.3	110.6	95.7	95.8

1 mean and standard deviation (SD) of eight replicates of the control group and 4 replicates of the treatment groups

2 Coefficient of Variation

3 formula: mean number of juveniles per treatment group * 100 / mean number of juveniles per control group

- = not applicable

The most recent non-GLP test with the reference item boric acid was performed at test concentrations of 44, 67, 100, 150 and 225 mg boric acid/kg soil dry weight. Boric acid showed an EC_{50} of 96 mg test item/kg soil dry weight (95 % confidence limits from 87 mg to 105 mg boric acid/kg soil dry weight) for reproduction according Probit analysis using maximum likelihood regression. The result is in the recommended range of the test guideline (about 100 mg boric acid/kg soil dry weight). The

$NOEC_{reproduction}$ was calculated to be 44 mg boric acid/kg soil dry weight and accordingly the $LOEC_{reproduction}$ was 67 mg boric acid/kg soil dry weight. Sufficient sensitivity of the test organisms was therefore demonstrated.

III. Conclusion

Over 4 weeks *Folsomia candida* were exposed to Prothioconazole + Spiroxamine EC 460 (160+300) G incorporated into soil to assess effects on mortality and reproduction. Due to an absence of effects an LC_{50} and EC_{50} could not be calculated. The $NOEC_{reproduction}$ was reported to be ≥169 mg test item/kg soil dry (equivalent to 51.0 mg spiroxamine/kg dry weight soil and 26.0 mg prothioconazole/kg dry weight soil, respectively). The $LOEC_{reproduction}$ was reported to be 169 mg test item/kg soil dry weight.

Assessment and conclusion by applicant:

This study has not been previously evaluated.

Validity criteria according to the most recent OECD 232 guideline (2016) were assessed and have been met:

- Mean adult mortality <20% at the end of the test (actual: 5%);
- The mean number of juveniles per vessel ≥100 at the end of the test (actual: 217);
- The coefficient of variation calculated for the number of juveniles <30% (actual: 7.6%).

The reference substance also demonstrated sufficient sensitivity of the test organisms.

The study is therefore considered acceptable.

The $NOEC_{reproduction}$ was reported to be ≥169 mg test item/kg soil dry (equivalent to 51.0 mg spiroxamine/kg dry weight soil and 26.0 mg prothioconazole/kg dry weight soil, respectively).

The results from this study have been statistically re-analysed and a summary of these results is presented below.

Data Point:	KCP 10.4.2.1/04
Report Author:	[redacted]
Report Year:	2020
Report Title:	Calculation of EC10 and EC20 values for Folsomia candida with prothioconazole + spiroxamine EC 460 G in a reproduction study
Report No:	047836-ECO20
Document No:	M-761556-01-1
Guideline(s) followed in study:	None
Deviations from current test guideline:	None
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	not applicable
Acceptability/Reliability:	Yes

Executive Summary

The report M-368461-01-1 on the effects of prothioconazole + spiroxamine EC 460 G in a springtail (*Folsomia candida*) reproduction study did not provide estimates of EC_{10} or EC_{20} values. Therefore, these values have been calculated in accordance with the Annex to Com. Reg. 284/2013. Due to the lack of a significant dose response and the lack of effects above 10% when compared to the control, it was

not possible to determine EC_{10} or EC_{20} values for reproduction. However, EC_{10} and EC_{20} values were estimated to be above the test rate of 169 mg/kg dws.

I. Methods

The statistical evaluation was performed with statistical software ToxRat Standard v3.3.0. Due to the lack of a significant dose response on the reproduction and due to the lack of effects above 10% when compared to the control, it was not possible to calculate EC_{10} and EC_{20} values.

II. Results

The re-calculation of the NOEC value for reproduction revealed no statistical significant effects when compared to the control at all test item treatment groups. Therefore, the NOEC for reproduction was determined to be the test item treatment at a rate of 169 mg/kg dws.

Due to the lack of a significant dose response on the reproduction and due to the lack of effects above 10% when compared to the control, it was not possible to calculate EC_{10} and EC_{20} values. Therefore, EC_{10} and EC_{20} values are estimated to be above the test rate of 169 mg/kg dws.

III. Conclusion

Due to the lack of a significant dose response and the lack of effects above 10% when compared to the control, it was not possible to determine EC_{10} or EC_{20} values for reproduction. However, EC_{10} and EC_{20} values were estimated to be above the test rate of 169 mg/kg dws.

Assessment and conclusion by applicant:

The statistical re-evaluation of the reproduction data could not determine reliable EC_{10} and EC_{20} values.

The NOEC of 169 mg/kg dws remains the critical endpoint from this study.

The conclusions made in the re-evaluation work are considered to be fully valid.

Data Point:	KCP 10.4.2 L/05
Report Author:	[redacted]
Report Year:	2011
Report Title:	Prothioconazole + spiroxamine EC 460 (160+300) G: Influence on the reproduction of the collembolan species Folsomia candida tested in artificial soil
Report No:	ERM-COLL-117 M1
Document No:	M-404668-01-1
Guideline(s) followed in study:	OECD 232 adopted September 07, 2009: OECD Guidelines for Testing Chemicals "Collembolan Reproduction Test in Soil
Deviations from current test guideline:	Yes – minor. Due to mistake the pH-values at the end of the study were not determined. No influence on the study, because the pH-values at study start were in the range recommended by the guideline.
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

Collembola (*Folsomia candida*) aged 10 to 12 days were exposed to Prothioconazole + Spiroxamine EC 460 (160+300) G incorporated into soil in a 4-week study to assess effects of reproduction.

Test organisms were exposed to 62.5, 125, 250, 500 and 1000 mg test item/kg soil and to a water control. Boric acid was used as a reference test in accordance with OECD 232 (2009) guidelines.

A statistically significant reduction in number of juveniles compared to the control was observed in the treatment groups with 250, 500 and 1000 mg test item/kg soil dry weight, resulting in reductions of 50.8, 94.1 and 99.0%, respectively.

$NOEC_{reproduction}$ and $LOEC_{reproduction}$ were 125 and 250 mg test item/kg soil dry weight, respectively.

The LC_{50} (adult mortality) and EC_{50} (reproduction) were 413 and 247 mg test item/kg soil dry weight, respectively.

I. Materials and Methods

Materials

Test Material	Prothioconazole + Spiroxamine EC 460 (160+300) G (PTZ+SPX EC 460 (160+300) G)
Lot/Batch #:	EDFL0001V7
Purity:	a) Prothioconazole: 151.1 g/L b) Spiroxamine: 296.3 g/L
Description:	Clear liquid, dark yellow
Stability of test compound:	Not reported
Reanalysis/Expiry date:	13 July 2011
Density:	0.981 g/mL
Treatments	
Test rates:	62.5, 125, 250, 500, 1000 mg test item/kg soil dry weight
Solvent/vehicle:	Water
Analysis of test concentrations:	No
Test organisms	
Species:	*Folsomia candida*, Collembola, Isotomidae
Source:	Bred at Bayer CropScience. Strain originally obtained from Ibacon, Institute for Analytic and Consulting, GmbH, D – 64380 Rossdorf.
Feeding:	Approximately 2 mg granulated dry yeast at the start of the study and after 14 days.
Treatment for disease:	None reported
Test design	
Test vessel:	Reusable glass vessels (volume 140 ml, diameter 5 cm at the bottom, height 7 cm). The test vessels were covered with glass lids.

Test medium:	Artificial soil according to OECD 232 (2009). 5% peat content
Replication:	8 control replicates and the 4 treatment replicates of each concentration
No. animals/vessel:	10
Duration of test:	28 days
Environmental test conditions	
Temperature:	20 ± 2°C
Photoperiod:	16 h light : 8 h dark at 608-624 lux

Study Design

Collembola (*Folsomia candida*) were exposed to Prothioconazole + Spiroxamine EC 460 (160+300) G over 4 weeks to assess the inhibition of reproduction.

The Collembola were 10 to 12 days old at the start of the study. For each replicate, 10 of the juvenile Collembola were placed in the test vessels, which had been prepared with the test item solution mixed with artificial soil. The soil was aligned with OECD 232 (2009) standard and approximately 30 g (wet weight) of the test substrate was filled in each test vessel, avoiding compression. Water was added until 50% water holding capacity was achieved.

The artificial soil was kept at 18 to 22°C, with the temperature continuously recorded by a thermo hygrograph integrated in the climatic chamber. The test vessels were exposed to 608 to 624 lux under a photoperiod of 16 h light: 8 h dark, monitored by an integrated luxmeter of the climatic chamber.

Five replicates were exposed to control (water) treatment, and to 62.5, 125, 250, 500 and 1000 mg test item/kg soil dry weight. During the study, the test organisms were fed with granulated dry yeast.

The most recent reference test with the toxic standard, boric acid, was conducted with doses at 44, 67, 100, 150 and 225 mg boric acid/kg artificial soil dry weight.

After 14 days, water content was checked and replenished if water loss exceeded 2% of initial content. Food was also checked at this time and the Collembola were fed again if necessary. Mortality and reproduction were reported after 28 days and were determined by the number of dead adult Collembola and the number of living juveniles detected using digital images.

II. Results and Discussion

Validity criteria according to the OECD 232 guideline to which the study was conducted were met:

- Mean adult mortality <20% at the end of the test (actual: 5%);
- The mean number of juveniles per vessel >100 at the end of the test (actual: 1570);
- The coefficient of variation calculated for the number of juveniles <30% (actual: 12%).

In the control group 5% of the adult Collembola died and at the maximum dose (1000 mg test item/kg soil dry weight) 90% of the adult Collembola died. The LC_{50} value, determined by probit analysis, was 413 mg test item/kg soil dry weight (95 % confidence limit 307 – 556 mg test item/kg soil dry weight).

Table CP 10.4.2.1/05-1 Survival of Adult Collembola after 4 weeks treatment (n=10/replicate)

	Treatment (mg test item/kg soil dry weight)					
	Control	**62.5**	**125**	**250**	**500**	**1000**
Mean[1]	9.5	8.5	9.3	7.5	3.5	1.0

	Treatment (mg test item/kg soil dry weight)					
	Control	**62.5**	**125**	**250**	**500**	**1000**
SD[1]	0.5	1.7	1.5	3.3	1.9	1.2
% mortality[2]	5.0	15.0	7.5	25.0	65.0	90.0

[1] mean and standard deviation (SD) of 8 replicates of the control group and 4 replicates for the treatment groups

[2] formula: ((initial placed organisms per vessel – mean of surviving adults per vessel) / 10) * 100

A statistically significant effect (Bonferroni-U-t test, one-sided-smaller, p = 0.05) was found in the treatment groups from 250 to 1000 mg test item/kg soil dry weight. There was no significant difference between control and the treatment groups with 62.5 and 125 mg test item/kg soil dry weight.

The No-Observed-Effect-Concentration ($NOEC_{reproduction}$) was 125 mg test item/kg soil dry weight. The Lowest-Observed-Effect-Concentration ($LOEC_{reproduction}$) was 250 mg test item/kg soil dry weight. The EC_{50} for reproduction, determined by probit analysis, was 247 mg test item/kg soil dry weight (95 % confidence limit 162 – 371 mg test item/kg soil dry weight).

Table CP 10.4.2.1/05-2 Reproduction of the Collembola after 4 weeks treatment (juveniles/replicate)

	Treatment (mg test item/kg soil dry weight)					
	Control	**62.5**	**125**	**250**	**500**	**1000**
Mean[1]	1569.6	1371.2	1451.8	773.5	92.8	15.5
SD[1]	188.3	92.2	179.2	295.2	24.6	19.7
CV[2]	12.0			-	-	-
% of control[3]	-	87.4	92.5	49.3*	5.9*	1.0*

[1] mean and standard deviation (SD) of five replicates
[2] Coefficient of Variation
[3] formula: mean number of juveniles per treatment group * 100 / mean number of juveniles per control group
- = not applicable
* = significantly different compared to the control (Dunnett's Test, one-sided-smaller, $\alpha = 0.05$)

The most recent non-GLP test with the reference item boric acid was performed at test concentrations of 44, 67, 100, 150 and 225 mg boric acid/kg soil dry weight. Boric acid showed an EC_{50} of 91 mg test item/kg soil dry weight (95 % confidence limits from 80 mg to 104 mg boric acid/kg soil dry weight) for reproduction according Probit analysis using maximum likelihood regression. The result is in the recommended range of the test guideline (about 100 mg boric acid/kg soil dry weight). The $NOEC_{reproduction}$ was calculated to be 44 mg boric acid/kg soil dry weight and accordingly the $LOEC_{reproduction}$ was 67 mg boric acid/kg soil dry weight. Sufficient sensitivity of the test organisms was therefore demonstrated.

III. Conclusion

Over 4 weeks *Folsomia candida* were exposed to Prothioconazole + Spiroxamine EC 460 (160+300) G incorporated into soil to assess effects on mortality and reproduction. The $NOEC_{reproduction}$ was determined to be 125 mg test item/kg soil dry weight (equivalent to 37.8 mg spiroxamine/kg dry weight soil and 19.3 mg prothioconazole/kg dry weight soil, respectively). The $LOEC_{reproduction}$ value was determined to be 250 mg test item/kg soil dry weight.

The LC_{50} (adult mortality) was 413 mg test item/kg soil dry weight. The EC_{50} (reproduction) was 247 mg test item/kg soil dry weight.

Assessment and conclusion by applicant:

This study has not been previously submitted or evaluated.

Validity criteria according to the most recent version of the OECD 232 guideline (2016) were assessed and have been met:

- Mean adult mortality <20% at the end of the test (actual: 5%);
- The mean number of juveniles per vessel ≥100 at the end of the test (actual: 1570);
- The coefficient of variation calculated for the number of juveniles <30% (actual: 12%).

The reference substance also demonstrated sufficient sensitivity of the test organisms.

The study is therefore considered acceptable.

The $NOEC_{reproduction}$ was determined to be 125 mg test item/kg soil dry weight (equivalent to 37.8 mg spiroxamine/kg dry weight soil and 19.3 mg prothioconazole/kg dry weight soil, respectively).

The results from this study have been statistically re-analysed and a summary of these results is presented below.

Data Point:	KCP 10.4.2.1/06
Report Author:	[redacted]
Report Year:	2020
Report Title:	Calculation of EC10 and EC20 values for Folsomia candida with prothioconazole + spiroxamine EC 460 G in a reproduction study
Report No:	047836-ECO21
Document No:	M-761557-01-1
Guideline(s) followed in study:	None
Deviations from current test guideline:	None
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	not applicable
Acceptability/Reliability:	Yes

Executive Summary

The report M-404668-01-1 on the effects of prothioconazole + spiroxamine EC 460 G in a springtail (*Folsomia candida*) reproduction study did not provide estimates of EC_{10} or EC_{20} values. Therefore, these values have been calculated in accordance with the Annex to Com. Reg. 284/2013. The resulting EC_{10} and EC_{20} values of 133.73 (95%CL: 77.09 – 232.00) and 164.98 (95%CL: 109.78 – 247.94) mg/kg dws, respectively, are considered reliable as the criteria for goodness of fit were met.

I. Methods

The statistical evaluation was performed with statistical software ToxRat Standard v3.3.0. To calculate ECx values, probit analysis using linear maximum likelihood regression was performed along with 95% ECx confidence limits based on normal approximation.

II. Results

The criteria for goodness of fit were met as the $P(Chi^2)$ value was 0.992, showing no significant deviation between fit and data, and a statistically significant concentration/response was found (p(F) = 0.029) for this parameter.

The resulting EC_{10} and EC_{20} values and the respective confidence intervals are presented in the following table.

Table CP 10.4.2.1/06-1 Results of the Probit analysis (max. likelihood regression) with reproduction at 28 d: Selected effective concentrations (EC_x) of the test item and their 95%-confidence limits (by normal approximation)

Parameter	Reproduction at test end (28 days)	
	EC_{10} (95 % confidence interval) [mg/kg dws]	EC_{20} (95 % confidence interval) [mg/kg dws]
Effect on reproduction	133.73 (77.09 – 232.00)	164.98 (109.78 – 247.94)

The resulting EC_{10} and EC_{20} values of 133.73 (95% CL: 77.09 – 232.00) and 164.98 (95% CL: 109.78 – 247.94) mg/kg dws, respectively, for springtail (*Folsomia candida*) in a Prothioconazole + Spiroxamine EC 460 G reproduction test (28 days period) are therefore considered reliable as the criteria for goodness of fit were met.

III. Conclusion

The resulting EC_{10} and EC_{20} values of 133.73 (95% CL: 77.09 – 232.00) and 164.98 (95% CL: 109.78 – 247.94) mg/kg dws, respectively, are considered reliable as the criteria for goodness of fit were met.

Assessment and conclusion by applicant:

The statistical re-evaluation of the reproduction data has determined an EC_{10} of 134 mg/kg dws.

As the NOEC is lower than the EC_{10}, the NOEC of 125 mg/kg dws shall be used in the risk assessment as the most critical endpoint from this study.

The values determined in the re-evaluation work are considered to be fully valid.

Data Point:	KCP 10.4.2.1/07
Report Author:	[redacted]
Report Year:	2020
Report Title:	1st final report amendment - Spiroxamine EC 500: Effects on reproduction of the predatory mite Hypoaspis aculeifer in artificial soil
Report No:	143091089
Document No:	M-688129-01-1
Guideline(s) followed in study:	Regulation (EC) No 1107/2009 (2009) OECD 226: Guidelines for the testing of chemicals - Predatory Mite (Hypoaspis (Geolaelaps) aculeifer) reproduction test in soil, adopted July 29, 2016
Deviations from current test guideline:	Nneo
Previous evaluation:	No, not previously submitted -
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

Adult *Hypoaspis aculeifer* were exposed to Spiroxamine EC 500 in a 14-day study to assess the effect on mortality and reproduction.

Hypoaspis aculeifer were exposed in artificial soil to a control and to test concentrations of 25, 50, 100, 200 and 400 mg test item/kg dry weight soil, according to guidelines set out in OECD 226 (2016). Dimethoate was used as a toxic standard.

The NOEC and LOEC values for mortality were determined to be ≥400 and >400 mg test item/kg dry weight soil, respectively.

The NOEC and LOEC values for reproduction were determined to be 200 and 400 mg test item/kg dry weight soil, respectively.

The EC_{50} value was estimated to be >400 mg test item/kg dry weight soil.

I. Materials and Methods

Materials

Test Material Spiroxamine EC 500

Lot/Batch #: EM4L018425

Purity: Spiroxamine EC 500: 50.0% w/w, corresponding to 501.6 g/L

Description: Yellow liquid

Reanalysis/Expiry date: 09 May 2020

Density: 1.004 g/mL

Treatments

Test rates: 25, 50, 100, 200 and 400 mg test item/kg dry weight soil

Test organisms

Species: *Hypoaspis aculeifer*, predatory mite, Laelapidae

Source: Ibacon GmbH, 64380 Rossdorf, Germany

Feeding:	One spatula of cheese mites (*Tyrophagus putrescentiae*) at test initiation and on test days 2, 5, 7, 9 and 12
Test design	
Test vessel:	Glass containers (volume: 100 mL; diameter: 5 cm) with tight screw top lids
Test medium:	Artificial soil. 5% peat content
Replication:	8 replicates for the control, 4 replicates per treatment group and 1 additional container per treatment to test the pH and water content of the test substrate at test termination
No. animals/vessel:	10 per test vessel
Duration of test:	14 days
Environmental test conditions	
Temperature:	18 - 22°C
pH:	6.0 – 6.2
Photoperiod:	16 hours light: 8 hours dark (at 400 – 800 lux)

Study Design

This study was conducted in order to assess the effects on reproduction of Spiroxamine EC 500 on *Hypoaspis aculeifer* over 14 days.

Ten adult female *Hypoaspis aculeifer* per replicate (8 replicates for the control group and 4 replicates for each treatment group) were exposed to control and treatments. Concentrations of 25, 50, 100, 200 and 400 mg test item/kg dry weight soil were mixed into the artificial soil. The test soil was composed of 74.8% fine quartz-sand, 20% kaolin clay, 5% sphagnum peat and 0.2% calcium carbonate. The soil was prepared according to the guideline OECD 226 (2016).

During the test, *Hypoaspis aculeifer* were fed with cheese mites (*Tyrophagus putrescentiae*) and kept in ventilated glass vessels. Temperatures of 18 - 22°C and a light regime of 400 – 800 Lux, 16 hour light: 8 hour dark were maintained throughout the test in a controlled environment chamber.

A reference test with the toxic standard, BAS 152 11 I (a.s. dimethoate), was performed at least once a year to ensure that the laboratory test conditions are adequate and to verify that the response of the test organisms does not change significantly over time.

Water content was checked 7 days after application by reweighing the additional test vessels. If the water loss exceeded 2% of the initial water content, the missing amount of water was added to all vessels of the treatment group.

Reproduction data were observed at test termination. Juveniles were counted twice under binocular microscopes. Statistical analysis was performed on the reproduction data using Shapiro-Wilk's test and Levene's test ($\alpha = 0.01$) to test for normal distribution and homogeneity. Since the reproduction data were normally distributed and homogeneous, further statistical analysis was performed using Williams t-test (multiple comparison, $\alpha = 0.05$, one-sided smaller).

Mortality data were observed at test termination. Missing adult mites were assumed dead and degraded. Statistical analysis was performed on the mortality data using Fisher's Exact Binomial Test (multiple comparison, with Bonferroni Correction, $\alpha = 0.05$, one-sided greater).

The software used to perform the statistical analysis was ToxRat Professional, Version 3.3.0, ToxRat® Solutions GmbH.

II. Results and Discussion

Validity criteria according to the OECD 226 guideline (2016), to which the study was conducted, were met.

- Mean adult female mortality should not exceed 20% at the end of the test (actual: 0%)
- The mean number of juveniles per replicate (with 10 adult females introduced) should be at least 50 at the end of the test (actual: 196 to 237)
- The coefficient of variation calculated for the number of juvenile mites per replicate should not be higher than 30% at the end of the definitive test (actual: 5.9%)

Mortality of *Hypoaspis aculeifer* in the test item treated groups ranged from 0% to 3%. The values were not statistically significantly different compared to the control (Fisher's Exact Test, $\alpha = 0.05$, one-sided greater). The NOEC and LOEC values were determined to be ≥400 and >400 mg test item/kg dry weight soil, respectively.

Table CP 10.4.2.1/07-1 Mortality data observed after 14 days exposure

Treatment group (mg test item/kg artificial soil dry weight soil)	Mean mortality (%)	Standard deviation	Significance[1]
Control	0	± 0	-
25	3	± 5	n.s.
50	3	± 5	n.s.
100	3	± 5	n.s.
200	3	± 5	n.s.
400	0	± 0	n.s.

[1]Fisher's Exact Test, one-sided greater, $\alpha = 0.05$

- not applicable

n.s. not significantly different compared to the control

There were no statistically significant effects on reproduction of *Hypoaspis aculeifer* up to and including the test concentration of 200 mg test item/kg dry weight soil (Williams t-test, $\alpha = 0.05$, one-sided smaller). At the concentration of 400 mg test item/kg dry weight soil a statistically significant decrease of reproduction was observed. The NOEC and LOEC values were determined to be 200 and 400 mg test item/kg dry weight soil, respectively.

Table CP 10.4.2.1/07-2 Reproduction data observed after 14 days exposure

Treatment group (mg test item/kg artificial soil dry weight soil)	Mean	Standard deviation	% of control	Significance[1]
Control	220	± 13	-	-
25	210	± 12	95	n.s.
50	187	± 7	85	n.s.
100	216	± 12	99	n.s.
200	213	± 19	97	n.s.

Treatment group (mg test item/kg artificial soil dry weight soil)	Mean	Standard deviation	% of control	Significance[1]
400	200	± 10	91	*

[1]Williams t-test, α = 0.05, one-sided smaller
- not applicable
n.s. not significantly different compared to the control
* significantly different compared to the control

The reference item dimethoate showed statistically significant treatment related effects on reproduction at a concentration of 2.23 mg dimethoate/kg soil and above. The EC_5 for reproduction was 2.47 mg dimethoate/kg soil. The EC_{50} determined in the reference test is slightly below the recommended range given in the test guideline (3.0 - 7.0 mg a.s./kg soil), however the results are considered to confirm that the test organisms at this test facility are sensitive to the effects of the reference substance and therefore the results achieved in this study are considered to be valid. The range of the past seven reference tests was between 2.47 to 4.12 mg a.s./kg soil.

III. Conclusion

Spiroxamine EC 500 caused no statistically significant effects on mortality of *Hypoaspis aculeifer* up to and including the test concentration of 400 mg test item/kg dry weight soil. Therefore, the NOEC and LOEC values for mortality were determined to be ≥400 and >400 mg test item/kg dry weight soil, respectively.

The NOEC and LOEC values for reproduction were determined to be 200 and 400 mg test item/kg dry weight soil, respectively (equivalent to 100 and 200 mg a.s./kg dry weight soil, respectively).

EC_x values could not be determined by statistical analysis since there was no adequate concentration response, therefore no EC_{10}/EC_{20} value can be reported. However, the EC_{50} was estimated to be >400 mg test item/kg dry weight soil.

Assessment and conclusion by applicant:

Validity criteria according to the most recent OECD 226 guideline (2016), to which the study was conducted, were met:

- Mean adult female mortality should not exceed 20% at the end of the test (actual: 0%)
- The mean number of juveniles per replicate (with 10 adult females introduced) should be at least 50 at the end of the test (actual: 196 to 237)
- The coefficient of variation calculated for the number of juvenile mites per replicate should not be higher than 30% at the end of the definitive test (actual: 5.9%)

The reference substance was also considered to demonstrate sufficient sensitivity of the test organisms.

The study is therefore considered acceptable.

The NOEC value for reproduction was determined to be 200 mg test item/kg dry weight soil (equivalent to 100 mg a.s./kg dry weight soil).

Data Point:	KCP 10.4.2.1/08
Report Author:	[redacted]
Report Year:	2012
Report Title:	Spiroxamine EC 500E G: Influence on mortality and reproduction on the soil mite species Hypoaspis aculeifer tested in artificial soil
Report No:	KRA-HR-69/12
Document No:	M-443019-01-1
Guideline(s) followed in study:	OECD 226 from October 03, 2008: OECD guideline for the Testing of Chemicals - Predatory mite (Hypoaspis (Geolaelaps) aculeifer) reproduction test in soil US EPA OCSPP: None
Deviations from current test guideline:	None
Previous evaluation:	No, not previously submitted -
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The purpose of the study was to assess the effects of Spiroxamine EC 500E G on mortality and reproduction on the soil mite species *Hypoaspis aculeifer* tested during an exposure of 14 days in artificial soil comparing control and treatment.

Ten adult, fertilized, female *Hypoaspis aculeifer* per replicate (8 control replicates and 4 replicates for each test item concentration) were exposed to control and treatments. Concentrations of 100, 178, 316, 562 and 1000 mg test item/kg dry weight artificial soil were tested.

The No Observed Effect Concentration (NOEC) was calculated to be ≥ 1000 mg test item/kg dry weight artificial soil. The Lowest Observed Effect Concentration (LOEC) > 1000 mg test item/kg dry weight artificial soil.

I. Materials and Methods

Materials

Test Material Spiroxamine EC 500E G

Lot/Batch #:	EDFL013642
Purity:	Analysed content(s) of a.s.: 508.1 g/L corresponding to 50.5 % w/w
Description:	Liquid, yellow-brown
Stability of test compound:	Sufficient based on expiration date
Reanalysis/Expiry date:	08 August 2014
Density:	1.006 g/ml

Treatments

Test rates:	100, 178, 316, 562 and 1000 mg test item/kg dry weight artificial soil
Solvent/vehicle:	Deionised water
Analysis of test concentrations:	No

Test design

Test species:	*Hypoaspis aculeifer*
Test vessel:	Reusable glass vessels (Weck Mini-Sturzglas, volume 140 mL, diameter 5 cm at the bottom, height 7 cm)
Test substrate:	5% Sphagnum-peat, 20% Kaolin clay, 74.7% fine quartz-sand, 0.3% Calcium carbonate
Replication:	Eight control replicates and four replicates for each test item concentration
No. of animals/vessel:	Ten
Duration of test:	14 days (plus two days for extraction)
Environmental test conditions	
Temperature:	20 ± 2°C
pH:	Test start: 6.19 to 6.38 Test end: 6.19 to 6.88
Photoperiod:	16 h light : 8 h dark (400 – 800 lux)
Water content:	47.43% to 52.7% of WHC_{max}

Study Design

The purpose of the study was to assess the effects of Spiroxamine EC 300E G on mortality and reproduction on the soil mite species *Hypoaspis aculeifer* tested during an exposure of 14 days in artificial soil comparing control and treatment.

Nominal test concentrations were 100, 178, 316, 562 and 1000 mg test item/kg dry weight artificial soil.

Ten adult female mites were added to each of the four replicate test vessels (eight for the control). Test vessels were reusable glass vessels (Weck Mini-Sturzglas, volume 140 mL, diameter 5 cm at the bottom, height 7 cm), filled with approximately 20 g dry weight artificial soil dry weight.

Directly after the addition of the *Hypoaspis aculeifer*, they were fed with cheese mites (*Tyrophagus putrescentiae*). Cheese mites were bred on brewers yeast in the laboratory. During the continuation of the test the soil mites were fed 3, 7 and 10 days after test start with the cheese mites.

The vessels were kept in a temperature-controlled room at 20 ± 2 °C under a 16-hour light to 8-hour darkness photo period. The light intensity at light period was between 400 - 800 Lux.

The surviving adults and living juveniles were counted as described under bioassay procedure. Missing adults (compared to the number of initially placed test organisms) were considered to be dead, since dead mites cannot be extracted.

The transfer of the test animals was finished within two hours after the application of the test item. After a period of 14 days, the surviving adults and the living juveniles per test vessel were extracted, applying a temperature gradient. The content of each test vessel was carefully transferred to sieve vessels (mesh size approximately 0.8 mm). Each sieve vessel was put onto another vessel containing a fixing liquid. The vessels were positioned in MCFADYEN-Extractor. The temperature was increased from approximately 25 to 40 °C within two days. Extracted mites were collected in a fixing solution (20 % ethylene glycol, 80 % deionised water; 2 g detergent/L fixing solution were added). The extracted mites in the fixing solution were stored in a refrigerator until the start of the counting of surviving adults and juveniles. All *Hypoaspis aculeifer* (adult females and juveniles) were counted under a binocular.

The surviving adults and living juveniles were counted as described under bioassay procedure. Missing adults (compared to the number of initially placed test organisms) were considered to be dead, since dead mites cannot be extracted.

Endpoints of the test were mortality of the adult, female *Hypoaspis aculeifer* in comparison to the initially placed test organisms expressed in % and the number of offspring hatched from the eggs and surviving until the end of the test period per test vessel (reproduction).

For the determination of normal distribution and homogeneity of variance Kolmogorroff-Smirnov Test and Cochran-Test ($\alpha = 0.05$), respectively were used. Data of reproduction were normally distributed and homogeneity of variances was given. Therefore William´s t-test for homogeneous variances (one-sided smaller, $\alpha = 0.05$) was used to determine NOEC and LOEC values. The software used to perform the statistical analysis was ToxRat Pro 2.10.

II. Results and Discussion

Validity criteria according to the guideline to which the study was conducted were met.

- Mean adult female mortality in the controls must not exceed 20% (actual: 5.0%)
- The mean number of juvenile mites per replicate to be at least 50 (actual: 326.8)
- The coefficient of variation for reproduction to be ≤30% (actual: 15.1%)

In the control group 5.0 % of the adult *Hypoaspis aculeifer* died which is below the allowed maximum of ≤20 % mortality. The LC_{50} could not be calculated and is considered to be >1000 mg test item/kg dry weight artificial soil.

Table CP 10.4.2.1/08-1 Survival of adult female *Hypoaspis aculeifer* after 14 days

Adults/vessel	Control	Treatment (mg test item/kg dry weight artificial soil)				
		100	178	316	562	1000
Replicate 1	6	9	10	8	10	10
Replicate 2	10	10	10	8	10	10
Replicate 3	10	10	10	9	9	9
Replicate 4	10	10	10	10	10	9
Replicate 5	10					
Replicate 6	10					
Replicate 7	10					
Replicate 8	10					
Mean	**9.5**	**9.8**	**10.0**	**8.8**	**9.8**	**9.5**
Standard deviation	**1.4**	**0.5**	**0.0**	**1.0**	**0.5**	**0.6**
Coefficient of variation	**14.9**	**5.1**	**0.0**	**10.9**	**5.1**	**6.1**
% Mortality	**5.0**	**2.5**	**0.0**	**12.5**	**2.5**	**5.0**

Concerning the number of juveniles statistical analysis (William´s t-test, one-sided smaller, $\alpha = 0.05$) revealed no significant differences between control and any concentration tested.

Therefore, the No-Observed-Effect-Concentration (NOEC) for reproduction was ≥1000 mg test item/kg dry weight artificial soil. The Lowest-Observed-Effect-Concentration (LOEC) for reproduction was >1000 mg test item/kg dry weight artificial soil. An EC_{50} for reproduction could not be calculated and is considered to be >1000 mg test item/kg dry weight artificial soil.

Table CP 10.4.2.1/08-2 Reproduction of *Hypoaspis aculeifer* after 14 days after test start (Juveniles/replicate)

Adults/vessel	Control	Treatment (mg test item/kg dry weight artificial soil)				
		100	178	316	562	1000
Replicate 1	218	299	370	256	311	363
Replicate 2	309	378	372	308	325	386
Replicate 3	316	359	310	331	346	357
Replicate 4	350	320	330	363	376	340
Replicate 5	374					
Replicate 6	349					
Replicate 7	332					
Replicate 8	366					
Mean	**326.8**	**339.0**	**345.5**	**314.5**	**339.5**	**361.5**
Standard deviation	**49.4**	**36.0**	**30.6**	**45.1**	**28.3**	**19.0**
Coefficient of variation	**15.1**	**10.6**	**8.8**	**14.3**	**8.3**	**5.3**
% Mortality	**100**	**103.7**	**105.7**	**96.3**	**103.9**	**110.6**

The reference item dimethoate produced an LC_{50} of 3.894 mg a.s./kg for mortality and an EC_{50} of 6.62 mg a.s./kg for reproduction. The EC_{50} determined in the reference test is within the recommended range given in the test guideline (3.0 - 7.0 mg a.s./kg soil) therefore the results are considered to demonstrate sufficient sensitivity of the test organism.

III. Conclusion

The No Observed Effect Concentration (NOEC) was calculated to be ≥ 1000 mg test item/kg dry weight artificial soil (equivalent to 505 mg a.s./kg soil). The Lowest Observed Effect Concentration (LOEC) > 1000 mg test item/kg dry weight artificial soil.

Assessment and conclusion by applicant:

Validity criteria according to the most recent version of the OECD 226 guideline (2016) were met.

- Mean adult female mortality in the controls must not exceed 20% (actual: 5.0%)
- The mean number of juvenile mites per replicate to be at least 50 (actual: 326.8)
- The coefficient of variation for reproduction to be ≤30% (actual: 15.1%)

The reference substance was also considered to demonstrate sufficient sensitivity of the test organisms.

The study is therefore considered acceptable.

The NOEC was determined to be 1000 mg test item/kg dry weight artificial soil (equivalent to 505 mg a.s./kg soil).

EC_{10} and EC_{20} values have not been determined as part of this study. However, it is very clear from the results that there was no treatment-related effect whatsoever. In fact, the number of juveniles produced was slightly greater in the majority of treatment groups when compared to the control. For

this reason it is considered that no EC_{10} or EC_{20} value would be determinable and the data have not been subject to statistical re-evaluation.

Data Point:	KCP 10.4.2.1/09
Report Author:	[redacted]
Report Year:	2017
Report Title:	Prothioconazole + spiroxamine EC 460 (160+300) G: Influence on mortality and reproduction of the soil mite species Hypoaspis aculeifer tested in artificial soil
Report No:	E 428 05070-6
Document No:	M-611272-01-1
Guideline(s) followed in study:	EU Directive 91/414/EEC Regulation (EC) No. 1107/2009 OECD Guideline 226 (2016) US EPA OCSPP Not Applicable
Deviations from current test guideline:	None
Previous evaluation:	No, not previously submitted -
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

Hypoaspis aculeifer were exposed to Prothioconazole + spiroxamine EC 460 (160+300) G in a 14-day study to assess the effects on mortality and reproduction.

Test organisms were exposed in artificial soil to a control and to concentrations of 18, 32, 56, 100, 178, 316, 562 and 1000 mg test item/kg dry weight soil, according to guidelines set out in OECD 226 (2016).

The NOEC and LOEC for adult mortality were ≥1000 and >1000 mg test item/kg dry weight soil, respectively.

The NOEC and LOEC for reproduction were 316 and 562 mg test item/kg dry weight soil, respectively.

The EC_{10} and EC_{20} for reproduction were 212 and 329 mg test item/kg dry weight soil, respectively.

I. Materials and Methods

Materials

Test Material	Prothioconazole + spiroxamine EC 460 (160+300) G (PTZ+SPX EC 460 (160+300) G)
Lot/Batch #:	2017-0002377-01
Purity:	Prothioconazole: 160.1 g/L (16.3% w/w) Spiroxamine: 294.2 g/L (29.9% w/w)
Description:	Yellow, dark, light turbid liquid
Stability of test compound:	Not reported
Reanalysis/Expiry date:	26 January 2017
Density:	0.985 g/ml

Treatments

Test rates:	18, 32, 56, 100, 178, 316, 562 and 1000 mg test item/kg dry weight artificial soil
Solvent/vehicle:	Water
Analysis of test concentrations:	No

Test organisms

Species:	*Hypoaspis aculeifer* (Acari: Laelapidae)
Source:	Bred at Bayer AG, the strain was originally obtained from ECT Oekotoxikologie GmbH, 65439 Floersheim am Main.
Acclimatisation period:	-
Feeding:	Fed with nematodes (*Panagrellus redivivus*) 3, 7 and 10 days after test start

Test design

Test vessel:	Reusable glass vessels (volume 140 mL, diameter 5cm at the bottom, height 7 cm). The test vessels were covered with glass lids to prevent *Hypoaspis aculeifer* from escaping but allowing aeration during the test period.
Test medium:	Artificial soil, 5% peat content.
Replication:	For the control 8 replicates and for the treatment groups 4 replicates
No. animals/vessel:	10
Duration of test:	14 days

Environmental test conditions

Temperature:	20 ± 2°C
Photoperiod:	16 h light: 8 h dark

Study Design

This study was conducted in order to assess the effects on reproduction and mortality of PTZ+SPX EC 460 (160+300) G on *Hypoaspis aculeifer* over 14 days.

Ten adult, fertilized female *Hypoaspis aculeifer* per replicate (8 replicates for the control group and 4 replicates for each treatment group) were exposed to control and treatments. Concentrations of 18, 32, 56, 100, 178, 316, 562 and 1000 mg test item/kg dry weight artificial soil were mixed into the artificial soil.

During the test, the *Hypoaspis aculeifer* were fed with nematodes and kept in reusable glass vessels covered with glass lids to prevent the test organisms from escaping but allowing aeration during the test period. During the study a temperature of 20 ± 2 °C and a light regime of 400 - 800 Lux, 16 h light : 8 h dark were maintained. The artificial soil was prepared according to the guideline.

After a period of 14 days, the surviving adults and the living juveniles were extracted by applying a temperature gradient using a MacFadyen-apparatus. Extracted mites were collected in a fixing solution (20% ethylene glycol, 80% deionised water; 2 g detergent/L fixing solution were added). All *Hypoaspis aculeifer* were counted under a binocular.

The corresponding non-GLP-test with the reference item dimethoate was performed at test concentrations of 1.0, 1.8, 3.2, 5.6 and 10.0 mg dimethoate/kg dry weight artificial soil.

II. Results and Discussion

Validity criteria according to the OECD 226 guideline (2016), to which the study was conducted, were met. In the control:

- Mean adult female mortality <20% (actual: 3.8%);
- The mean number of juveniles per replicate (with 10 mites introduced) >50 (actual: 358.9);
- The coefficient of variation calculated for the number of juvenile mites per replicate <30% (actual: 4.8%).

In the control group 3.8% ofthe adult *Hypoaspis aculeifer* died which is below the allowed maximum of < 20% mortality. Concerning the mortality of the adult test organisms statistical analysis (Fisher's Exact Binomial Test with Bonferroni Correction, one-sided greater, a = 0.05) revealed no significant difference between control and any treatment group up to and including 1000 mg test item/kg dry weight soil. Therefore, the No-Observed-Effect-Concentration (NOEC) for mortality is >1000 mg test item/kg dry weight soil. The Lowest-Observed-Effect-Concentration (LOEC) for mortality is >1000 mg test item/kg dry weight soil.

Concerning the number of juveniles, statistical analysis (William's t-test, one-sided smaller, a = 0.05) revealed no significant difference between control and any treatment group up to and including 316 mg test item/kg dry weight soil. Therefore, the No-Observed-Effect-Concentration (NOEC) for reproduction is 316 mg test item/kg dry weight soil. The Lowest-Observed-Effect-Concentration (LOEC) for reproduction is 562 mg test item/kg dry weight soil. The EC_{10} for reproduction was calculated to be 212 (95% confidence limits: 107 - 296) mg test item/kg soil dry weight and the EC_{20} was calculated to be 329 (95% confidence limits: 212 - 418) mg test item/kg soil dry weight.

Table CP 10.4.2.1/09-1 Mortality, Reproduction and mean number of juveniles per replicate of *Hypoaspis aculeifer* exposed to PTZ+SPX EC 460 (160+300) G

Treatment (mg test item/kg dry weight soil)	Adult mortality (%)	Mean number juveniles per replicate ± S.D.	Reproduction (% of control)
Control	3.8	358.9 ± 17.1	-
18	12.5	344.8 ± 19.2	96.1
32	5.0	365.0 ± 20.2	101.7
56	2.5	361.5 ± 20.5	100.7
100	5.0	369.0 ± 24.7	102.8
178	5.0	371.3 ± 26.0	103.4
316	3.3	327.0 ± 17.1	91.1
562	5.0	160.8 ± 69.4	44.8*
1000	10.0	176.0 ± 47.7	49.0*

* statistically significant

A non-GLP test with the reference item dimethoate was performed at test concentrations of 1.0, 1.8, 3.2, 5.6 and 10.0 mg dimethoate/kg dry weight soil. Dimethoate showed an LC_{50} of 2.8 mg a.s./kg soil for mortality of the adult mites according Probit analysis (confidence limits from 1.8 mg a.s./kg to 4.3 mg a.s./kg soil). The reproduction of the soil mites was not significantly reduced in comparison to the control up to and including 3.2 mg a.s./kg dry weight soil. Therefore the NOEC was calculated to be 3.2 mg a.s./kg dry weight soil and accordingly the LOEC was 5.6 mg a.s./kg dry weight soil. Dimethoate EC 400 G showed an EC_{50} of 5.2 mg a.s./kg dry weight soil (95 % confidence limits from 4.3 mg a.s./kg

soil to 6.0 mg a.s./kg soil) for reproduction according Weibull analysis using maximum likelihood regression. This is in the recommended range of the guideline, indicating that an EC_{50} based on the number of juveniles of 3.0 - 7.0 mg a.s./kg dry weight soil shows that the test organisms are sufficiently sensitive.

III. Conclusion

Hypoaspis aculeifer were exposed to Prothioconazole + spiroxamine EC 460 (160+300) G in a 14-day study to assess the effects on mortality and reproduction.

The NOEC and LOEC for adult mortality were ≥1000 and >1000 mg test item/kg dry weight soil, respectively.

The NOEC for reproduction was 316 mg test item/kg dry weight (equivalent to 94.5 mg spiroxamine/kg dry weight soil and 51.5 mg prothioconazole/kg dry weight soil, respectively). The LOEC for reproduction was 562 mg test item/kg dry weight soil.

The EC_{10} for reproduction was 212 mg test item/kg dry weight soil (equivalent to 63.4 mg spiroxamine/kg dry weight soil and 34.6 mg prothioconazole/kg dry weight soil, respectively) and the EC_{20} for reproduction was 329 mg test item/kg dry weight soil.

Assessment and conclusion by applicant:

This study has not been previously submitted or evaluated.

Validity criteria according to the OECD 226 guideline (2016), to which the study was conducted, were met. In the control:

- Mean adult female mortality <20% (actual: 3.8%);
- The mean number of juveniles per replicate (with 10 mites introduced) >50 (actual: 358.9);
- The coefficient of variation calculated for the number of juvenile mites per replicate <30% (actual: 4.8%).

The reference substance also demonstrated sufficient sensitivity of the test organisms.

The study is therefore considered acceptable.

The NOEC was determined to be 316 mg test item/kg soil dry weight. The EC_{10} for reproduction was calculated to be 212 mg test item/kg dry weight soil (equivalent to 63.4 mg spiroxamine/kg dry weight soil and 34.6 mg prothioconazole/kg dry weight soil, respectively). The EC_{10} was lower than the NOEC value and therefore has been taken as the critical endpoint from this study.

CP 10.4.2.2 Higher tier testing

No data are available. Field data with Prothioconazole & Spiroxamine EC 460 are not considered necessary as an acceptable risk following the proposed uses has been demonstrated using the available laboratory data.

CP 10.5 Effects on soil nitrogen transformation

The available soil nitrogen transformation data for spiroxamine and the metabolites of spiroxamine are summarized in the table below.

Table CP 10.5-1 Summary of nitrogen transformation studies with metabolies of spiroxamine

Test item	Test type	Endpoints		Reference
Spiroxamine EC 500	Nitrogen transformation	**<25% effect after 42 days at 10.0 mg/kg soil (5.0 mg a.s./kg soil)**	NEW	M-680703-01-1
KWG 4168-desethyl (M01)	Nitrogen transformation	**<25% effect after 28 days at 4.53 mg/kg soil**	EU	M-282056-01-1
KWG 4168-despropyl (M02)	Nitrogen transformation	**<25% effect after 70 days at 5.0 mg/kg soil**	NEW	M-680757-01-1
KWG 4168-N-oxide (M03)	Nitrogen transformation	**<25% effect after 56 days at 6.9 mg/kg soil**	NEW	M-680759-01-1
KWG 4168-acid (M06)	Nitrogen transformation	**<25% effect after 28 days at 5.0 mg/kg soil**	NEW	M-688312-01-1

EU: previously evaluated as part of the original EU review and listed in EFSA conclusion and DAR
NEW: new study or data generated since the previous EU review or previously not submitted
Values in **bold** have been used in the risk assessment

The available soil nitrogen and carbon transformation data for prothioconazole, prothioconazole-desthio and prothioconazole-S-methyl are summarised in the table below.

Table CP 10.5-2 Summary of nitrogen and carbon transformation studies with prothioconazole, prothioconazole-desthio and prothioconazole-S-methyl

Test item	Test type	Endpoints		Reference
Prothioconazole	Nitrogen transformation	<25% effect after 28 days at 2.0 kg a.s./ha	EU	EFSA Conclusion[1]
Prothioconazole	Carbon transformation	<25% effect after 28 days at 2.0 kg a.s./ha	EU	
Prothioconazole-desthio	Nitrogen transformation	<25% effect after 28 days at 1.0 kg/ha	EU	
Prothioconazole-desthio	Carbon transformation	<25% effect after 28 days at 0.2 kg/ha	EU	
Prothioconazole-S-methyl	Nitrogen transformation	<25% effect after 28 days at 2.0 kg/ha	EU	
Prothioconazole-S-methyl	Carbon transformation	<25% effect after 28 days at 2.0 kg/ha	EU	

EU: previously evaluated as part of the original EU review and listed in EFSA conclusion and DAR
[1] EFSA Scientific Report (2007) 106, 1-98. Conclusion on the peer review of Prothioconazole
Values in **bold** have been used in the risk assessment

The available soil nitrogen transformation data for Prothioconazole + Spiroxamine EC 460 are summarized in the table below.

Table CP 10.5-3 Summary of nitrogen transformation studies with Prothioconazole + Spiroxamine EC 460

Test item	Test type	Endpoints		Reference
Prothioconazole + Spiroxamine EC 460	Nitrogen transformation	**<25% effect after 28 days at 12.5 L/ha (16.42 mg product/kg soil)**	NEW	M-619760-01-1

EU: previously evaluated as part of the original EU review and listed in EFSA conclusion and DAR
NEW: new study or data generated since the previous EU review or previously not submitted
Values in **bold** have been used in the risk assessment

Toxicity endpoints

Nitrogen transformation data for spiroxamine technical are not available therefore the study conducted using Spiroxamine EC 500 has been submitted and can be used to represent the toxicity of spiroxamine. Note that the data generated with the representative formulation, Prothioconazole + Spiroxamine EC 460 are considered to provide the most relevant endpoints for the risk assessment of this representative formulation.

For the toxicity endpoints of prothioconazole and the associated metabolites the endpoints have been taken directly from the 2007 EFSA Conclusion without any further consideration. Risk assessments for prothioconazole have been presented here but only for completeness and to allow for the risk assessment of this representative formulation, containing spiroxamine, to be conducted. Discussion of the specific endpoints for prothioconazole are not considered to be part of the Renewal of Approval for spiroxamine.

Exposure

Full details of the PEC_{soil} calculations have been provided in Document M-CP Section 9 Environmental Fate. The maximum initial and accumulation PEC_{soil} values for spiroxamine and its metabolites, as calculated using FOCUS equations, are given in the table below for the application rate of 375 g a.s./ha.

Table CP 10.5-4 PEC_{soil} for spiroxamine and its metabolites

Substance	1 x 375 g a.s./ha		2 x 375 g a.s./ha	
	Max PEC_{soil} (mg/kg)	**$PEC_{soil\ accumulation}$ (mg/kg)**	**Max PEC_{soil} (mg/kg)**	**$PEC_{soil\ accumulation}$ (mg/kg)**
Cereals				
Spiroxamine	0.100	0.035	**0.181**	0.069
M01	0.011	0.015	0.022	**0.030**
M02	0.008	0.011	0.016	**0.021**
M03	0.008	0.010	0.017	**0.022**
M06	0.006	0.013	0.012	**0.025**

PEC_{soil} values used in the risk assessment are highlighted in **bold**

For the risk assessment below, the risk envelope approach has been applied in which the PEC_{soil} values for the proposed use with the highest application rate has been used. Thus, the risk assessment has been conducted for the use on cereals at 2 x 1.25 L/ha. For spiroxamine the maximum initial PEC_{soil} value was higher than the accumulation value therefore the maximum initial PEC_{soil} value has been used in the assessment. However, for all of the spiroxamine metabolites the $PEC_{soil\ accumulation}$ values were greater than the maximum initial PEC_{soil} values therefore the risk assessment for the metabolites has been conducted using the worst case $PEC_{soil\ accumulation}$ values.

For Prothioconazole + Spiroxamine EC 460 the formulation PEC_{soil} was determined to be 0.328 mg/kg soil for the maximum application rate of 1.25 L/ha. Please refer to Document M-CP Section 9 Environmental Fate for further details.

For prothioconazole, prothioconazole-desthio and prothioconazole-S-methyl, PEC_{soil} values of 0.081, 0.075 and 0.021, respectively have been used in the risk assessment. These values have been taken directly from the spiroxamine draft RAR (Spiroxamine dRAR, Volume 3, Annex B.9) and are considered to cover the uses proposed for Prothioconazole + Spiroxamine EC 460.

Isomers

For parent spiroxamine the environmental fate soil degradation data currently suggest that there is no significant selective degradation of isomers over time. As a result, the toxicity data generated using the mixture of the isomers (*i.e.* Spiroxamine EC 500) are considered to represent the toxicity of the isomers in the ratios that would occur in the soil following application. In accordance with the isomer Guidance Document[17] it is therefore not necessary to apply any additional Uncertainty Factor (UF) to the risk assessment (*i.e.* a UF of 1.0 is used).

For the metabolites of spiroxamine there are no chiral data available to be able to make this assessment therefore there is a possibility that selective degradation of isomers could occur in the soil over time. In order to account for any possible increased toxicity to soil organisms as a result of an increase in the ratio of a single isomer, an UF has been applied to the risk assessment of M01, M02, M03 and M06. The UF have been calculated following the recommendations of the isomer Guidance Document and have been presented in the table below.

Table CP 10.5-5 Uncertainty Factors determined for the nitrogen transformation data with the metabolites of spiroxamine

Test item	Study reference	Test material batch number	Isomer ratio	UF [1]
Spiroxamine	-	-		1.0 [2]
M01	M-282056-01-1	921103ELB02	A:B 56:42	4.76
M02	M-680757-01-1	AE 1344303-PU-00	A:B 83.1:16.0	12.5
M03	M-680759-01-1	M26999	D1:D2:D3:D4 22:21:26:31	9.52
M06	M-688317-01-1	AE 1344313-01-03	A:B 47:53	4.26

[1] Changes in stereoisomeric excess are unknown therefore Uncertainty Factor = 100/content of lowest stereoisomer (%) used for ecotox endpoint [as indicted in Table B.1, p.30 of isomer GD] and assumes that the toxicological effects of the mixture can be attributed to a single isomer. This assumes that all enantiomer ratios can be safely assumed to be 50:50. For example A:B ratio of 83.1:16 would be 100/(16/2) = UF of 12.5

[2] No additional UF required for parent as no significant change in isomeric ratios has been demonstrated

Risk assessment

The effect concentrations for spiroxamine, prothioconazole, Prothioconazole + Spiroxamine EC 460 and for the metabolites are compared to the PEC_{soil} values in the following table.

[17] Guidance of EFSA on risk assessments for active substances of plant protection products that have stereoisomers as components or impurities and for transformation products of active substances that may have stereoisomers. EFSA Journal 2019;17(8):5804

Table CP 10.5-6 Soil micro-organism risk assessment for spiroxamine, prothioconazole, Prothioconazole + Spiroxamine EC 460 and relevant metabolites following application of Prothioconazole + Spiroxamine EC 460 to cereals

Intended use	Cereals 2 x 1.25 L/ha			
Test item	Endpoint	PEC_{soil}	UF[1]	Risk acceptable[2]
Prothioconazole + Spiroxamine EC 460	<25% effect after 28 days at 12.5 L/ha (16.42 mg product/kg soil)	0.328 mg product/kg soil	-	Yes
Spiroxamine EC 500	<25% effect after 42 days at 10.0 mg/kg soil (5.0 mg a.s./kg soil)	0.181 mg a.s./kg soil	1.0	Yes
M01	<25% effect after 28 days at 4.53 mg/kg soil	0.030 mg/kg soil	4.76	Yes
M02	<25% effect after 70 days at 5.0 mg/kg soil	0.021 mg/kg soil	12.5	Yes
M03	<25% effect after 56 days at 6.9 mg/kg soil	0.022 mg/kg soil	9.52	Yes
M06	<25% effect after 28 days at 5.0 mg/kg soil	0.025 mg/kg soil	4.26	Yes
Prothioconazole	<25% effect after 28 days at 2.03 kg a.s./ha (2.71 mg/kg soil)	0.081 mg a.s./kg soil	-	Yes
Prothioconazole-desthio	<25% effect after 28 days at 1.0 kg/ha (1.33 mg/kg soil)	0.075 mg/kg soil	-	Yes
Prothioconazole-S-methyl	<25% effect after 28 days at 2.02 kg/ha (2.69 mg/kg soil)	0.021 mg/kg soil	-	Yes

[1] Uncertainty Factor applied to account for the unknown effect of a possible change in isomer ratios over time
[2] Risk assessment has compared the NOEC against the $PEC_{soil} \times UF$
- Not applicable

Prothioconazole + Spiroxamine EC 460 had no significant effect on soil micro-organisms at concentrations up to 16.42 mg product/kg soil. This is higher than the maximum PEC_{soil} of 0.328 mg product/kg soil following the worst-case application to cereals. Thus, the margins of safety in the risk assessment is a factor of 50 for Prothioconazole + Spiroxamine EC 460. This supports the conclusion that under field conditions, the proposed uses of Prothioconazole + Spiroxamine EC 460 pose no unacceptable risk to non-target soil micro-organisms.

In addition, no significant effects (>25%) were shown in the studies with Spiroxamine EC 500, M01, M02, M03, M06, prothioconazole, prothioconazole-desthio and prothioconazole-S-methyl at concentrations greatly exceeding the predicted soil concentrations. Acceptable risks to non-target soil micro-organisms from exposure to spiroxamine, prothioconazole and the metabolites, following application of Prothioconazole + Spiroxamine EC 460, have therefore also been demonstrated.

Biodiversity

No relevant scientifically peer-reviewed open literature could be found on spiroxamine or its major metabolites, from an ecotoxicological perspective, on soil micro-organisms. Therefore, it is considered that the potential impact of the active substance on biodiversity and the ecosystem, including potential indirect effects *via* alteration of the food web, are covered by the risk assessment for soil micro-organisms in this section.

With respect to the risk assessments for soil micro-organisms, which demonstrated an acceptable outcome with large margins of safety and without the need for risk mitigation, the applicant concludes that the use of the representative lead formulation (Prothioconazole + Spiroxamine EC 460) has a low potential to cause unacceptable effects on biodiversity and the ecosystem *via* trophic interactions. To the best of our knowledge and with the presented safety profile of the spiroxamine a.s., metabolites and the representative lead formulation, the applicant does not foresee any unacceptable effects on biodiversity and the ecosystem with spiroxamine.

Summaries of the available soil micro-organism studies have been presented below.

Data Point:	KCP 10.5/01
Report Author:	[redacted]
Report Year:	2020
Report Title:	Spiroxamine EC 500: Effects on the activity of the soil microflora in the laboratory (nitrogen transformation)
Report No:	143091080
Document No:	M-680763-01-1
Guideline(s) followed in study:	None
Deviations from current test guideline:	Storage temperature of soil extracts (no effect)
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The purpose of this study was to assess the effects of the test item on the activity (nitrogen transformation) of soil microflora in the laboratory.

Spiroxamine EC 500 was tested at concentrations of 2.0 and 10 mg test item/kg soil dry weight.

The test item SPX EC 500 had no impact on nitrogen transformation (nitrate content, mineral nitrogen content and nitrate formation rates) of soil microorganisms when applied at 2.0 mg and 10 mg test item/kg soil dry weight treatment.

I. Materials and Methods

Materials

Test Material Spiroxamine EC 500

Lot/Batch #: EM4L098425

Active ingredients: Spiroxamine (KWG 4168): 50.0% w/w, corresponding to 501.6 g/L

Description: Yellow liquid

Stability of test compound: Not reported

Reanalysis/Expiry date: 09 May 2020

Density: 1.004 g/mL

Treatments

Test rates:	1 and 5 mg a.s./kg (corresponding to 2.0 and 10 mg SPX EC 500/kg)
Solvent/vehicle:	Ultrapure water
Analysis of test concentrations:	None

Test design

Test vessel:	500 mL plastic boxes containing 300 g dw soil
Test soil:	A loamy sand
Source:	In der Speyerer Hohl, No. 977
Replication:	Three per control and test group
Duration of test:	42 days

Environmental test conditions

Temperature:	20 ± 2 °C
pH:	7.2 to 7.5
Moisture:	48 to 50% of maximum water holding capacity
Photoperiod:	Constant darkness

Study Design

The purpose of this study was to assess the effects of the test item on the activity (nitrogen transformation) of soil microflora in the laboratory.

Triplicate samples of each soil (containing 300 g dry weight (dw)) were tested.

The soil batch used in this study was according to the guideline and was taken from fallow grassland, where no pesticides or organic or mineral fertiliser had been used on the soil for at least four years prior to test initiation. The soil was collected from Rhineland Palatinate district authority, Mechtersheim Germany municipality and the location was "In der Speyerer Hohl ", No. 977". The soil was a loamy sand.

The water content of one replicate of each treatment group was determined at each sampling date. Water losses were compensated by adding ultrapure water. Throughout the study, the water content ranged from 48% to 50% WHC. The pH was determined at test start and on day 28 in one replicate of each treatment group. Over the course of the study, the pH value was between 7.2 and 7.5.

All solvents or chemicals used were of analytical grade or higher purity. The lucerne meal used was fine powdered lucerne green grass meal; the analysed carbon and nitrogen content was 40.9% and 2.7%, respectively. The ratio of carbon to nitrogen was 15 / 1.

The test item was soluble in water; therefore a stock solution in ultrapure water was prepared by dissolving 44.9 mg SPX EC 500 in 50 mL ultrapure water and mixed into the soil by means of a laboratory mixer. Throughout the application the soil was ventilated and the soil water content was adjusted to 48% of WHC.

To the control, acetone treated quartz sand (evaporated) and additionally 0.5% lucerne meal (based on soil dry weight) was mixed into the soil. The soil water content was adjusted to 49% of WHC. The soil water content was determined in one replicate of each treatment group at each sampling.

For the determination of nitrogen content, soil samples were taken within 6 hours after application and afterwards on each sampling date (7, 14, 28 and 42 days). The nitrogen content was determined in each sample of treated and control soils.

For extraction, 24 g to 25 g soil were suspended in 100 mL 0.1 M KCl-solution and agitated for one hour. The suspension was centrifuged (Multifuge 3s+, 4350 rpm) and the extracts were stored deep frozen.

Amounts of 70.8 mg, 74.0 mg, and 72.2 mg ammonium sulfate, sodium nitrite and potassium nitrate, respectively, were diluted in 1000 mL (ammonium sulfate, sodium nitrite) and 100 ml (potassium nitrate) 0.1 M KCl to prepare the standard stock solutions for ammonium-N, nitrite-N and nitrate-N determination. Appropriate aliquots of the stock solutions were automatically diluted by the dilution unity with 0.1 M KCl to prepare 6 standard solutions at a range of 0.5 mg/L to 3.0 mg/L for ammonium-N and nitrite-N and 7 standard solutions at a range of 1.0 mg/L to 12.0 mg/L for nitrate-N determination. Before photometric determination, frozen soil extracts were thawed. For nitrite-N, nitrate-N and ammonium-N determination undiluted extracts were used. For determination undiluted extracts (days 0 to 28) and 1:2 in 0.1 M KCl diluted extracts (day 42) were used.

II. Results and Discussion

Validity criteria according to the OECD 216 (2000) guideline, to which the study was conducted, were met as the control variation between control replicates was less than ± 15% (maximum variation: 3.05%).

No adverse effects of the test item on nitrate content in soil were observed at day 28. At day 28, differences to the control were -1.35% and -12.62% in the 2 mg and 10 mg test item/kg soil dry weight treatment, respectively.

No adverse effects of the test item on nitrate content in soil were observed at test end at day 42. At day 42, differences to the control were -1.88% and -10.14% in the 2 mg and 10 mg test item/kg soil dry weight treatment, respectively.

At day 28 and 42, the difference was statistically significant compared to the control for the high test rate (Student t-test, $\alpha = 0.05$).

Very low nitrite and ammonium contents below 0.8 mg/kg dry weight were measured at day 28 and 42 in control and the test item treatments.

The mineral nitrogen contents in soil were within the trigger range of ± 25% at day 28. At day 28, differences to the control were -2.11% and -12.75% in the 2 mg and 10 mg test item/kg soil dry weight treatment, respectively.

The mineral nitrogen contents in soil were within the trigger range of ± 25% at day 42. At day 42, differences to the control were -1.88% and -10.01% in the 2 mg and 10 mg test item/kg soil dry weight treatment, respectively.

At day 28 and 42, the difference was statistically significant compared to the control for the high test rate (Student t-test, $\alpha = 0.05$), but within the trigger range.

Table CP 10.5.01-1 Nitrogen transformation test, effects of the test item on ammonium (mean values)

Days after treatment	Control		2 mg SPX EC 500/kg soil dw		10 mg SPX EC 500/kg soil dw	
	Ammonium	CV	Ammonium	Dev. %	Ammonium	Dev. %
0	6.771	1.70	6.484	-4.24	6.483	-4.25
7	1.354	4.95	1.277	-5.69	1.186	-12.41*
14	0.805	4.10	0.710	-11.80	0.984	22.24

Days after treatment	Control		2 mg SPX EC 500/kg soil dw		10 mg SPX EC 500/kg soil dw	
	Ammonium	CV	Ammonium	Dev. %	Ammonium	Dev. %
28	0.774	59.17	0.457	-40.96	0.594	-23.26
42	0.741	0.81	0.723	-2.43	0.704	-4.99

* Significantly different to the control (t-test at $p \leq 0.05$)

Table CP 10.5/01-2 Nitrogen transformation test, effects of the test item on nitrite (mean values)

Days after treatment	Control		2 mg SPX EC 500/kg soil dw		10 mg SPX EC 500/kg soil dw	
	Nitrite	CV	Nitrite	Dev. %	Nitrite	Dev. %
0	0.303	5.94	0.262	-13.53*	0.261	-14.88*
7	0.258	0.00	0.258	0.00	0.258	0.00
14	0.258	0.00	0.258	0.00	0.258	0.00
28	0.258	0.00	0.258	0.00	0.258	0.00
42	0.258	0.00	0.258	0.00	0.258	0.00

* Significantly different to the control (t-test at $p \leq 0.05$)

Table CP 10.5/01-3 Nitrogen transformation test, effects of the test item on nitrate (mean values)

Days after treatment	Control		2 mg SPX EC 500/kg soil dw		10 mg SPX EC 500/kg soil dw	
	Nitrate	CV	Nitrate	Dev. %	Nitrite	Dev. %
0	26.643	0.25	27.075	1.62*	27.017	1.40*
7	23.368	2.94	03.713	1.48	23.366	-0.01
14	26.363	3.05	26.293	-0.27	23.901	-9.34*
28	38.926	0.86	38.400	-1.35	34.013	-12.62*
42	49.628	0.98	48.694	-1.88	44.598	-10.14*

* Significantly different to the control (t-test at $p \leq 0.05$)

Table CP 10.5/01-4 Nitrogen transformation test, effects of the test item on N_{min} (mean values)

Days after treatment	Control		2 mg SPX EC 500/kg soil dw		10 mg SPX EC 500/kg soil dw	
	N_{min}	CV	N_{min}	Dev. %	N_{min}	Dev. %
0	33.717	0.58	33.821	0.31	33.767	0.15
7	24.980	2.50	25.248	1.07	24.810	-0.68
14	27.426	2.85	27.261	-0.60	25.143	-8.32*
28	39.958	1.43	39.116	-2.11	34.865	-12.75*

Days after treatment	Control		2 mg SPX EC 500/kg soil dw		10 mg SPX EC 500/kg soil dw	
	N_{min}	CV	N_{min}	Dev. %	N_{min}	Dev. %
42	50.627	0.96	49.674	-1.88	45.560	-10.01

* Significantly different to the control (t-test at $p \leq 0.05$)

Table CP 10.5/01-5 Nitrogen Transformation Test: Effects of the test item on Nitrate Formation Rates (Mean Values)

Interval[1]	Control		2 mg SPX EC 500/kg soil dw			10 mg SPX EC 500/kg soil dw		
	Mean mg NO_3-N/kg soil dry weight per day[2]							
Sampling days	mg/day	CV%	mg/day	Dev. %[4]	sig.[5]	mg/day	Dev. %[4]	sig.[5]
0 - 7	-0.468	-21.37	-0.480	2.56	n.s.	-0.522	11.54	n.s.
0 - 14	-0.020	-300.00	-0.056	180.00	n.s.	-0.223	1015.00	*
0 - 28	0.439	3.19	0.405	-7.74	n.s.	0.250	-43.05	*
0 - 42	0.547	2.01	0.515	-5.85	n.s.	0.419	-23.40	*
Interval[1]	Mean mg NO_3-N/kg soil dry weight per day[3]							
Sampling days	mg/day	CV%	mg/day	Dev. %[4]	sig.[5]	mg/day	Dev. %[4]	sig.[5]
0 - 7	-0.468	-21.37	-0.480	2.56	n.s.	-0.522	11.54	n.s.
7 - 14	0.428	12.62	0.368	-14.02	n.s.	0.076	-82.24	*
14 - 28	0.898	4.01	0.865	-3.67	n.s.	0.722	-19.60	*
28 - 42	0.764	6.28	0.735	-3.80	n.s.	0.756	-1.05	n.s.

[1]: Time interval
[2]: Calculated from the mean values of NO_3-N content between the sampling date and day 0
[3]: Calculated from the mean values of NO_3-N content between each sampling date
[4]: Deviation from control
[5]: sig.: Significance according Student-t-test, two sided, $\alpha = 0.05$ (* = significant; n. s.: not significant)
CV: Coefficient of variation (calculated as SD / mean value * 100)

The reference item sodium chloride was tested in a GLP study. Sodium chloride was tested at 16 g/kg soil dry weight. The variation of replicate control samples was less than 15%. The reference item had a retarding effect of more than ± 25% compared to the control at days 28 and 96 after application. The results of the study proved sensitivity of the test system and provided assurance that the laboratory test conditions are adequate.

III. Conclusion

After 42 days, the test item SPX EC 500 had no impact on nitrogen transformation (nitrate content, mineral nitrogen content and nitrate formation rates) of soil microorganisms when applied at 2.0 mg and 10 mg test item/kg soil dry weight treatment (equivalent to 1.0 and 5.0 mg a.s./kg soil dry weight, respectively).

Assessment and conclusion by applicant:

Validity criteria according to the OECD 216 (2000) guideline were met as the control variation between control replicates was less than ± 15% (maximum variation: 3.05%).

The reference item demonstrated sufficient sensitivity of the test system.

The study is therefore considered acceptable.

After 42 days, the test item had no impact on nitrogen transformation of soil microorganisms when applied at rates up to 10 mg test item/kg soil dry weight (equivalent to 5.0 mg a.s./kg soil dry weight, respectively).

Data Point:	KCP 10.5/02
Report Author:	[redacted]
Report Year:	2017
Report Title:	Prothioconazole + spiroxamine EC 460 (160+300) G: Effects on the activity of soil microflora (nitrogen transformation test)
Report No:	17 48 SMN 0029
Document No:	M-619760-01-1
Guideline(s) followed in study:	OECD 216, adopted January 21, 2000; OECD Guideline for the Testing of Chemicals, Soil Microorganisms: Nitrogen Transformation. EU Directive 91/414/EEC Regulation (EC) No 1107/2009 (2009) US EPA OCSPP Not Applicable
Deviations from current test guideline:	None
Previous evaluation:	No, not previously submitted -
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

A loamy sand soil was exposed to Prothioconazole + spiroxamine EC 460 (160+300) G for 28 days to assess the effects on soil nitrogen transformation.

Prothioconazole + spiroxamine EC 460 (160+300) G was applied at concentrations of 1.64 mg and 16.42 mg test item/kg soil dry weight. Application rates were equivalent to 1.25 L test item/ha and 12.5 L test item/ha. Controls and a reference item, Dinoterb, were used in this study.

No adverse effects of exposure to Prothioconazole + spiroxamine EC 460 (160+300) G on nitrogen transformation in soil were observed at either tested concentration.

I. Materials and Methods

Materials

Test Material Prothioconazole + spiroxamine EC 460 (160+300) G

Lot/Batch #: EV63001576

Purity: Prothioconazole: 160.1 g/L
Spiroxamine: 294.2 g/L

Description: Yellow dark liquid

Stability of test compound:	Not reported
Reanalysis/Expiry date:	26 January 2019
Density:	0.985 g/mL
Treatments	
Test rates:	1.64 and 16.42 mg test item/kg soil dry weight (1.25 and 12.5 L test item/ha equivalent)
Test design	
Test vessel:	Wide mouth glass flasks (500 mL) with screw caps
Solvent/vehicle:	Deionised water
Replication:	3 replicates
Duration of test:	28 days
Environmental test conditions	
Temperature:	18.9 - 21.2 °C
pH:	6.3
Photoperiod:	Darkness

Study Design

This study was conducted in order to assess the effects of Prothioconazole + spiroxamine EC 460 (160+300) G on soil nitrogen transformation over 28 days.

The soil was sourced from Wassergut Canitz, Germany, where plant protection products had not been applied since 1990. A reference item, Dinoterb, was tested routinely in a separate study to verify the sensitivity of the test system.

The experiment was carried out over a period of 28 days. The soil was exposed to a control consisting of deionised water only and to the test item at concentrations of 1.25 L test item/ha and 12.5 L test item/ha (equivalent to 1.64 and 16.42 mg test item/kg soil dry weight, respectively). Each treatment consisted of three replicates.

The soil was mixed with 0.5% Lucerne, the test item was mixed with deionised water and was subsequently mixed with the soil by means of a hand-stirrer. Water was added to the soil to achieve a water content of approximately 45% of water holding capacity.

The soils were incubated in wide mouth glass flasks (500 mL) for 28 days in darkness at 18.9 to 21.2 °C.

A sample of each replicate of each treatment was taken at intervals of 3 hours, 7, 14 and 28 days and the nitrogen transformation of the soil was determined.

II. Results and Discussion

Validity criteria according to the OECD 216 guideline (2000), to which the study was conducted, were met.

- Variation between replicate control samples ≤15% (actual 5.4%)

Exposure to Prothioconazole + spiroxamine EC 460 (160+300) G caused a temporary inhibition of the daily nitrate rate at the tested concentration of 1.64 mg test item/kg soil dry weight at time interval 7-14 days after application.

However, no adverse effects of Prothioconazole + spiroxamine EC 460 (160+300) G on nitrogen transformation in soil could be observed at both tested concentrations at the end of the test. Differences from the control of +10.9% (test concentration 1.64 mg test item/kg soil dry weight) and -7.1% (test concentration 16.42 mg test item/kg soil dry weight) were measured at the end of the 28-day incubation period.

No statistically significant differences to the control were observed in either test concentration at any time interval.

Table CP 10.5/02-1 Effects of exposure to Prothioconazole + spiroxamine EC 460 (160+300) on soil nitrogen transformation

mg test item/kg dry weight soil	Days after treatment	Mean mg Nitrate/kg dry weight soil	SD (mg Nitrate/kg dry weight soil)	CV (%)
Control	0	28.6	0.10	0.3
	7	57.2	1.41	2.5
	14	67.8	3.64	5.4
	28	82.8	1.85	2.2
1.64	0	28.7	0.46	-
	7	57.7	1.31	-
	14	64.5	2.67	-
	28	81.1	3.06	-
16.42	0	27.6	1.17	-
	7	59.2	0.85	-
	14	67.3	0.67	-
	28	81.3	0.82	-

Limit of quantification = LOQ: 0.84 mg/100 g soil d.w.
CV = Coefficient of Variation
SD = Standard Deviation

Table CP 10.5/02-2 Effects of exposure to Prothioconazole + spiroxamine EC 460 (160+300) on soil nitrogen transformation rate per day

mg test item/kg dry weight soil	Days after treatment	Mean mg N/kg dry weight soil	SD (mg N/kg dry weight soil)	Difference from control (%)
Control	0-7	4.09	0.21	-
	7-14	1.51	0.54	-
	14-28	1.07	0.34	-
1.64	0-7	4.14	0.2	+1.3

mg test item/kg dry weight soil	Days after treatment	Mean mg N/kg dry weight soil	SD (mg N/kg dry weight soil)	Difference from control (%)
	7-14	0.97	0.3	-35.8
	14-28	1.19	0.13	+10.9
16.42	0-7	4.51	0.28	+10.5
	7-14	1.16	0.22	-23.6
	14-28	1.00	0.03	-17.1

SD Standard deviation

In a separate study the reference item Dinoterb caused stimulations of nitrogen transformation of +35.4 %, +28.2 % and +126.8 % at 6.80, 13.60 and 27.20 mg Dinoterb per kg soil dry weight, respectively, determined 28 days after application, thereby demonstrating sufficient sensitivity of the test system.

III. Conclusion

Prothioconazole + spiroxamine EC 460 (160+300) caused no adverse effects (difference to control < 25 %) on the soil nitrogen transformation (expressed as NO_3-N production) at the end of the 28-day incubation period. The study was performed in a field soil at concentrations up to 16.42 mg test item/kg soil dry weight, which are equivalent to application rates up to 12.5 L test item/ha.

Assessment and conclusion by applicant:

This is a new study that has not been previously reviewed or evaluated.

Validity criteria according to the OECD 216 guideline (2000), to which the study was conducted, were met.

- Variation between replicate control samples ≤15% (actual 5.4%)

The reference item used was considered to demonstrate sufficient sensitivity of the test system.

The study is therefore considered acceptable.

There were <25% effects after 28 days at concentrations up to 16.42 mg test item/kg soil dry weight, which are equivalent to application rates up to 12.5 L test item/ha.

CP 10.6 Effects on terrestrial non-target higher plants

The available data for Prothioconazole + Spiroxamine EC 460 with non-target terrestrial plants are presented in the table below.

Table CP 10.6-1 Summary of non-target terrestrial plant studies with Prothioconazole + Spiroxamine EC 460

Organism	Test item	Test type	Endpoints		Reference
Lolium perenne [m] *Allium cepa* [m] *Brassica napus* [d] *Glycine max* [d] *Solanum lycopersicum* [d] *Beta vulgaris* [d]	Prothioconazole + Spiroxamine EC 460	21-day Seedling emergence	NOER 2.50 L product/ha ER_{50} >5.00 L product/ha	NEW	M-688131-01-1

Organism	Test item	Test type	Endpoints		Reference
Lolium perenne m *Allium cepa* m *Brassica napus* d *Glycine max* d *Solanum lycopersicum* d *Beta vulgaris* d	Prothioconazole + Spiroxamine EC 460	21-day Vegetative vigour	NOER 0.63 L product/ha **ER_{50} 4.22 L product/ha**	NEW	M-688315-01-1
Avena sativa m *Zea mays* m *Cucumis sativus* d *Brassica napus* d *Helianthus annuus* d *Glycine max* d	Prothioconazole + Spiroxamine EC 460	21-day Seedling emergence	**ER_{50} >1.25 L product/ha**	EU	M-182345-02-1
Avena sativa m *Zea mays* m *Cucumis sativus* d *Brassica napus* d *Lactuca sativa* d *Glycine max* d	Prothioconazole + Spiroxamine EC 460	21-day Vegetative vigour	**ER_{50} >1.25 L product/ha**	EU	M-182346-02-1

d: dicotylodonous; m: monocotyledonous
EU: previously evaluated as part of the original EU review and listed in EFSA conclusion and DAR
New: new study or data generated since the previous EU review or previously not submitted
Endpoints in **bold** have been used in the risk assessment

Prothioconazole endpoints

The EFSA Conclusion for prothioconazole (EFSA Scientific Report (2007) 106, 1-98) provides non-target terrestrial plant endpoints which have been conducted with both prothioconazole technical and a 250 EC formulation of prothioconazole which is not considered to be relevant for the risk assessment of Prothioconazole + Spiroxamine EC 460. The data generated using Prothioconazole + Spiroxamine EC 460 are considered to be the most relevant and have therefore been used in the risk assessment.

In order to assess the potential risks to non-target terrestrial plants, following exposure to Prothioconazole + Spiroxamine EC 460, it is considered most appropriate to use the toxicity data generated using this specific formulation. The data presented above have been generated using the representative formulation, Prothioconazole + Spiroxamine EC 460, and are therefore considered suitable for use in the risk assessment.

Risk assessment

The risk assessment has been conducted in accordance with the SANCO[18] terrestrial guidance document.

The lowest ER_{50} values were determined in the non-GLP seedling emergence and vegetative vigour studies in which there were less than 50% effects at the test rate of 1.25 L product/ha. As such, it is considered more appropriate to use the worst case bound ER_{50} value of 4.22 L product/ha determined in

[18] SANCO/10329/2002 rev 2 final (17 October 2002). Guidance Document on Terrestrial Ecotoxicology Under council Directive 91/414/EEC

the GLP vegetative vigour study. However, in order to present a conservative risk assessment, both ER_{50} values of >1.25 and 4.22 L product/ha have been used in the risk assessment below.

Exposure

Effects on non-target terrestrial plants are of concern in the off-field environment, where plants may be exposed to spray drift. The amount of spray drift reaching off-crop habitats is calculated using the appropriate percentile estimates, which depends on the number of applications, and is derived from the BBA (2000[19]) values from the spray-drift predictions of Ganzelmeier & Rautmann (2000[20]).

The worst case representative use of Prothioconazole + Spiroxamine EC 460 is for two applications to cereals at a maximum rate of 1.25 L product/ha. This use has been considered in the risk assessment below and covers all other representative uses of Prothioconazole + Spiroxamine EC 460.

The drift rate (predicted environmental rate, $PER_{off\text{-}field}$) associated with field crops (cereals) has been calculated based on spray drift predictions for one application using 90th percentile drift values and for two applications using 82nd percentile drift values. This gives drift rates of 2.77% at 1 m for field crops and 2.38% at 1 m for field crops for one and two applications, respectively. These equate to drift factors of 0.0277 and 0.0238 for one and two applications, respectively.

The calculated drift rates in L product/ha for the use on cereals are presented in the following table.

Table CP 10.6-2 Off-field drift rates following application of Prothioconazole + Spiroxamine EC 460

Crop	Maximum application rate (L product/ha)	Number of applications	Drift distance (m)	Drift factor	MAF	$PER_{off\text{-}field}$ (L product/ha)
Cereals	1.25	1	1	0.0277	1.0	0.0346
		2		0.0238	1.9*	0.0565

* Worst case MAF for two applications to soil substrate

The highest $PER_{off\text{-}field}$ value has been determined to be 0.0565 L product/ha and has therefore been used in the risk assessment below.

Isomers

In terms of organism exposure, the critical highest predicted concentrations in the environment will occur immediately or very shortly after application therefore the effects of potential changes in the isomer ratios over time in the environment are not considered to be relevant to the non-target terrestrial plant risk assessment. However, even if exposure to residues over a prolonged period of time were to occur, according to the current residues data set for spiroxamine there are no indications of a significant change in isomer ratios therefore no additional factor need be applied to the risk assessments below (*i.e.* an UF of 1.0 has been used).

Risk assessment for Terrestrial Non-Target Higher Plants

The risk to non-target plants in the off-crop environment from spray drift following application of Prothioconazole + Spiroxamine EC 460 has been assessed by comparing the ER_{50} values from seedling

[19] BBA (2000) Bundesanzeiger Jg. 52 (Official Gazette), Nr 100, S. 9879-9880 (25.05.2000) Bekanntmachung über die Abtrifteckwerte, die bei der Prüfung und Zulassung von Pflanzenschutzmitteln herangezogen werden. Public domain.

[20] Ganzelmeier H., Rautmann D. (2000) Drift, drift-reducing sprayers and sprayer testing. Aspects of Applied Biology 57, 2000, Pesticide Application. Public domain.

emergence and vegetative vigour effects with the highest $PER_{off\text{-}field}$ in order to calculate TER values according to the following equation.

$$TER = \frac{ER_{50}\ (\text{L product/ha})}{PER_{off\text{-}field}\ (\text{L product/ha})}$$

The TER values have been evaluated against the trigger value of 5 and are presented in the table below.

Table CP 10.6-3 Prothioconazole + Spiroxamine EC 460 TER values for non-target terrestrial plants

Crop	Effect	ER_{50} (L product/ha)	Application rate (L product/ha)	Off-field exposure			Trigger value
				Distance (m)	PER (L product/ha)	TER	
Cereals	Vegetative vigour	4.22	2 x 1.25	1	0.0563	74.7	5
	Seedling emergence & Vegetative vigour	1.25				>22.1	

Based on seedling emergence and vegetative vigour data, an acceptable risk to non-target terrestrial plants has been demonstrated following the proposed uses of Prothioconazole + Spiroxamine EC 460, with TER values in excess of the trigger value of 5. No further risk assessment is considered to be necessary.

Biodiversity

No relevant scientifically peer-reviewed open literature could be found on spiroxamine or its major metabolites, from an ecotoxicological perspective, on non-target terrestrial plants. Therefore, it is considered that the potential impact of the active substance on biodiversity and the ecosystem, including potential indirect effects *via* alteration of the food web, are covered by the risk assessment for non-target terrestrial plants in this section.

With respect to the NTTP off-field risk assessment, which demonstrated acceptable off-field risks without the need for risk mitigation, the applicant concludes that the use of the representative lead formulation (Prothioconazole + Spiroxamine EC 460) has a low potential to cause unacceptable effects on biodiversity and the ecosystem *via* trophic interactions. To the best of our knowledge and with the presented safety profile of the spiroxamine a.s. and the representative lead formulation, the applicant does not foresee any unacceptable effects on biodiversity and the ecosystem with spiroxamine.

CP 10.6.1 Summary of screening data

No screening data available. Please refer to Section CP 10.6.2 for seedling emergence and vegetative vigour data.

CP 10.6.2 Testing on non-target plants

Data Point:	KCP 10.6.2/03
Report Author:	[redacted]; [redacted]
Report Year:	2020
Report Title:	Prothioconazole + spiroxamine EC 460: Effects on terrestrial (non-target) plants: Seedling emergence and seedling growth test
Report No:	M-688131-01-1
Document No:	M-688131-01-1
Guideline(s) followed in study:	Regulation (EC) No 1107/2009 (2009) OECD Guideline for the Testing of Chemicals No. 208 "Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test" (adopted July 19, 2006)
Deviations from current test guideline:	None
Previous evaluation:	No, not previously submitted -
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The effect of Prothioconazole + Spiroxamine EC 460 on the seedling emergence and growth of monocot (perennial ryegrass, *Lolium perenne*; onion, *Allium cepa*) and dicot (oilseed rape, *Brassica napus*; soybean, *Glycine max*; tomato, *Solanum lycopersicum*; sugar beet, *Beta vulgaris*) crops was studied at nominal concentrations of 0.31, 0.63, 1.25, 2.50 and 5.00 L test item/ha.

The growth medium used in the test was sterilised soil (pH: 6.1 ± 0.4; organic carbon: 0.65 ± 0.08%).

All seeds were planted the day prior to test item application and the exposure time was either 14 or 21 days after 50% seedling emergence in the control depending on the growth of the seedlings. Spray treatments were once applied, at test initiation, at the nominal spray volume of 200 L/ha.

The emergence rate was not statistically significantly reduced for any of the species tested. Slight mortality was observed in oilseed rape and soybean at the test concentrations of 0.31 and 0.63 L test item/ha, respectively. The mortality was not statistically different to that of the control. There were slight phytotoxic effects observed in some test species including necrosis and chlorosis.

The most sensitive species in terms of fresh weight was *Beta vulgaris* with a NOER of 2.50 L test item/ha. All other species showed a NOER of ≥5.00 L test item/ha and a LOER of >5.00 L test item/ha. The ER_{50} for all test species was considered to be >5.00 L test item/ha.

I. Materials and Methods

Materials

Test Material Prothioconazole + Spiroxamine EC 460

Lot/Batch #: EM4L025614

Purity: Prothioconazole: 16.2% w/w (159.0 g/L)
Spiroxamine: 30.1% w/w (295.7 g/L)

Description: Yellow-brown liquid

Reanalysis/Expiry date: 08 April 2021

Density: 0.983 g/mL

Treatments

Test rates: Nominal: 0.31, 0.63, 1.25, 2.50 and 5.00 L test item /ha.

Test organisms

Species:	Perennial ryegrass (*Lolium perenne*), onion (*Allium cepa*), oilseed rape (*Brassica napus*), soybean (*Glycine max*), tomato (*Solanum lycopersicum*), sugar beet (*Beta vulgaris*)
Source:	Not reported

Test design

Test vessel:	Commercial plastic flower pots, 15 cm diameter
Test medium:	Sterilised soil at pH 6.1 ± 0.4; C_{org} 0.65 ± 0.08%
Replication:	4 – 10 pots per treatment group
No. /vessel:	At least 20 plants per treatment group
Duration of test:	21 days

Environmental test conditions

Temperature:	22°C ± 10°C
Relative humidity:	70% ± 25%
pH:	6.1 ± 0.4
Photoperiod:	16 hours light, 8 hours dark (light intensity minimum 200 µE/m²/s)

Study Design

This study was conducted in order to evaluate the effect of Prothioconazole + Spiroxamine EC 460 on the seedling emergence and growth of monocot and dicot crops.

Test species were monocotyledonous plants from two families (perennial ryegrass and onion) and dicotyledonous plants from four different families (oilseed rape, soybean, tomato and sugar beet).

Plants were grown in commercial plastic flower pot in a growth chamber at 22 ± 10 °C under a 16 hour light 8 hour dark photoperiod. Between four and ten replicates with twenty seeds per species were tested.

Sterilised sandy loam soil was used as the test medium.

At test initiation, spray solution made up of the test item dissolved in deionised water was applied to the soil surface using a spray chamber with an overhead nozzle (set at 40 cm above sprayed surface).

Observations of phytotoxicity were made on days 7, 14 and 21 according to the EPPO Standard 135 after 50% seedling emergence in the control. Fresh weights were determined 14 or 21 days after 50% seedling emergence in the control. Observations of mortality were made on days 7 and 14 or 7, 14 and 21 days after 50% seedling emergence in the control. Growth stages at day 14 or 21 after 50% seedling emergence in the control were recorded according to BBCH-Monograph – Growth stages.

Statistical analysis was carried out on the fresh weight data using the Shapiro-Wilk's test ($\alpha = 0.05$) and the Levene's test ($\alpha = 0.05$). The Dunnett's t-test (multiple comparison, one-sided smaller, $\alpha = 0.05$) was used if the data were normally distributed and homogeneous. For the mortality and emergence data, Fisher's Exact Binomial Test (with Bonferroni Correction, multiple comparison, one-sided greater, $\alpha = 0.05$) was used. The software used to perform the statistical analysis was ToxRat Professional, Version 3.3.0, ® ToxRat Solutions GmbH.

Analytical method

Samples of water were analysed using the validated analytical method M-688131-01-1, report reference M-688131-01-1 **(see Doc MCP Section 5)**.

II. Results and Discussion

Validity criteria according to the OECD 208 guideline (2006), to which the study was conducted, were met.

- The seedling emergence is at least 70% (actual: 95-100%)
- The seedlings do not exhibit visible phytotoxic effects and the plants exhibit only normal variation in growth and morphology for that particular species (actual: achieved)
- The mean survival of emerged control seedlings is at least 90% for the duration of the study (actual: 100%)
- Environmental conditions for a particular species are identical and growing media contain the same amount of soil matrix, support media, or substrate from the same source (actual: achieved)

The analytical recovery rate of the active substance prothioconazole in the stock solution was 110 % of the nominal value and 98 % of the nominal value for spiroxamine, respectively.

There were some statistically significant reductions in the observed fresh weight of sugar beet, which was significantly reduced at 5.00 L test item/ha. However, there were no statistically significant reductions in the observed fresh weight of oilseed rape, soybean, tomato, perennial ryegrass or onion. There were no statistically significant differences in emergence observed at any test concentration as compared to the control for any of the species tested.

Table CP 10.6.2/03-1 Summarised results of emergence, fresh weight and plant growth

Species	Treatment group (L test item/ha)	Emergence (%)	Fresh weight (g) after 21 days	Standard deviation	Effect[1] (%)	Growth stage (BBCH) after 21 days
Oilseed rape	Control	95	16.34	± 0.93	-	15-16
	0.31	95	15.53	± 1.59	5.0	15-16
	0.63	100	15.91	± 1.05	-2.6	14-15
	1.25	100	15.41	± 0.79	-5.7	15-16
	2.50	95	15.62	± 1.72	-4.4	14-16
	5.00	95	15.99	± 1.34	-2.1	14-16
Soybean	Control	95	7.58	± 2.08	-	12-13
	0.31	95	7.84	± 2.17	3.4	12-13
	0.63	95	6.25	± 2.11	-17.6	12-13
	1.25	100	6.40	± 2.49	-15.6	12-13
	2.50	90	7.80	± 1.45	2.9	12-13
	5.00	80	6.63	± 2.42	-12.6	12-13
Tomato	Control	95	3.99	± 1.17	-	12
	0.31	90	3.78	± 1.25	-5.4	12
	0.63	100	4.45	± 1.77	11.3	12
	1.25	90	3.89	± 2.01	-2.6	12
	2.50	100	3.50	± 0.96	-12.5	12
	5.00	90	2.83	± 0.79	-29.1	12
Sugar beet	Control	100	3.54	± 0.74	-	12
	0.31	95	3.69	± 0.89	4.1	12
	0.63	90	3.58	± 1.20	1.1	12
	1.25	100	3.63	± 0.75	2.6	12
	2.50	95	3.01	± 0.99	-15.0	12

Species	Treatment group (L test item/ha)	Emergence (%)	Fresh weight (g) after 21 days	Standard deviation	Effect[1] (%)	Growth stage (BBCH) after 21 days
	5.00	100	2.14	± 0.95	-39.5*	12
Perennial ryegrass	Control	95	9.41	± 2.53	-	21-22
	0.31	100	9.71	± 0.66	3.2	21-22
	0.63	95	8.30	± 1.90	-11.8	21-22
	1.25	80	8.48	± 1.30	-9.9	21-22
	2.50	90	8.57	± 0.83	-8.9	21-22
	5.00	95	9.33	± 1.44	-0.9	21-22
Onion	Control	100	2.46	± 0.62	-	11-12
	0.31	85	2.33	± 0.60	-5.4	11-12
	0.63	95	2.29	± 0.23	-7.0	11-12
	1.25	95	2.53	± 0.45	2.7	11-12
	2.50	90	2.60	± 0.42	5.4	11-12
	5.00	90	2.29	± 0.58	-6.9	11-12

[1] negative values indicate reduction compared to the control

* Statistically significantly different compared to the control

Slight mortality was observed for oilseed rape at the lowest test concentration of 0.31 L test item/ha (5%) and for soybean at 0.63 L test item/ha (16%). However, the mortality was not statistically significant as compared to the control. Slight phytotoxic effects were observed in all species tested. In addition, sugar beet and tomato showed leaf deformations, tomato showed chlorosis in one pot. All phytotoxic effects remained ≤30% in all species.

Table CP 10.6.2/03-2 Summarised results of mortality and phytotoxicity

Species	Treatment group (L test item/ha)	Mortality (%)	Phytotoxicity (%)		
			7 days	14 days	21 days
Oilseed rape	Control	0	0	0	-
	0.31	5	5	6	-
	0.63	0	0	0	-
	1.25	0	0	0	-
	2.50	0	0	4	-
	5.00	0	0	3	-
Soybean	Control	0	0	0	-
	0.31	0	4	1	-
	0.63	16	14	15	-
	1.25	0	14	13	-
	2.50	0	1	2	-
	5.00	0	10	5	-
Tomato	Control	0	0	0	-
	0.31	0	4	9	-
	0.63	0	2	2	-
	1.25	0	4	6	-
	2.50	0	3	13	-

Species	Treatment group (L test item/ha)	Mortality (%)	Phytotoxicity (%)		
			7 days	14 days	21 days
	5.00	0	2	18	-
Sugar beet	Control	0	0	0	-
	0.31	0	0	0	-
	0.63	0	0	5	-
	1.25	0	5	4	-
	2.50	0	1	6	-
	5.00	0	27	30	-
Perennial ryegrass	Control	0	0	0	0
	0.31	0	0	0	0
	0.63	0	0	3	3
	1.25	0	0	0	0
	2.50	0	0	0	0
	5.00	0	9	0	0
Onion	Control	0	0	0	0
	0.31	0	0	0	3
	0.63	0	10	0	0
	1.25	0	0	0	0
	2.50	0	0	5	0
	5.00	0	8	6	5

The most sensitive species was *Beta vulgaris* (based on fresh weight) with a NOER of 2.50 L test item/ha and a LOER of 5.00 L test item/ha. All other species showed a NOER of ≥5.00 L test item/ha and a LOER of >5.00 L test item/ha. The ER_{50} for all test species was considered to be >5.00 L test item/ha.

III. Conclusion

The most sensitive species was *Beta vulgaris* (based on fresh weight) with a NOER of 2.50 L test item/ha and a LOER of 5.00 L test item/ha. All other species showed a NOER of ≥5.00 L test item/ha and a LOER of >5.00 L test item/ha. The ER_{50} for all test species was considered to be >5.00 L test item/ha.

The emergence rate was not statistically significantly reduced for any species tested.

Mortality was observed for oilseed rape at the lowest concentration rate of 0.31 L test item/ha (5%) and for soybean at 0.63 L test item/ha (16%). The mortality was not statistically significantly different as compared to the control.

Phytotoxic effects observed were necrosis and growth reduction. In addition, sugar beet and tomato showed leaf deformations, tomato showed chlorosis in one pot. The phytotoxic effects remained ≤30% in all species.

The ER_{50} for all tested species was considered to be >5.00 L test item/ha.

Assessment and conclusion by applicant:

This is a new study that has not been previously submitted or evaluated.

Validity criteria according to the OECD 208 guideline (2006), to which the study was conducted, were met.

- The seedling emergence is at least 70% (actual: 95-100%)

- The seedlings do not exhibit visible phytotoxic effects and the plants exhibit only normal variation in growth and morphology for that particular species (actual: achieved)
- The mean survival of emerged control seedlings is at least 90% for the duration of the study (actual: 100%)
- Environmental conditions for a particular species are identical and growing media contain the same amount of soil matrix, support media, or substrate from the same source (actual: achieved)

Analytical verification of the stock solution used for the spray solutions confirmed correct dosing of the test system. The study is therefore considered acceptable.

The ER_{50} for all tested species was considered to be > 5.00 L test item/ha.

The NOER was determined to be 2.50 L test item/ha.

Data Point:	KCP 10.6.2/04
Report Author:	[redacted]
Report Year:	2020
Report Title:	Prothioconazole + spiroxamine EC 460: Effect on terrestrial (non-target) plants: Vegetative vigour test - 1st final report amendment
Report No:	143491087
Document No:	M-688315-01-1
Guideline(s) followed in study:	Regulation (EC) No 1107/2009 (2009) OECD Guideline for the Testing of Chemicals No. 227 "Terrestrial Plant Test: Vegetative Vigour Test" (adopted July 19, 2006)
Deviations from current test guideline:	None
Previous evaluation:	No, not previously submitted -
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The effect of Prothioconazole + Spiroxamine EC 460 on the vegetative vigour of monocot (perennial ryegrass, *Lolium perenne*; onion, *Allium cepa*) and dicot (oilseed rape, *Brassica napus*; soybean, *Glycine max*; tomato, *Solanum lycopersicum*; sugar beet, *Beta vulgaris*) was investigated at nominal test concentrations of 0.313, 0.63, 1.25, 2.50 and 5.00 L test item/ha.

The growth medium used in the test was sterilised soil (pH: 5.9 ± 0.5; organic carbon: 0.66 ± 0.07%). Plants were treated with foliar spray application at the 2-4 leaf stage.

Spray treatments were applied once at test initiation, at the nominal spray volume of 200 litres/ha. Plants were assessed for mortality and phytotoxicity on days 7, 14 and 21. At study termination, endpoint determinations were performed for plant dry weights.

There were some statistically significant reductions in the observed fresh weight of species including oilseed rape, soybean and sugar beet. However, there were no statistically significant reductions in the observed fresh weight of tomato, perennial ryegrass or onion.

There was no mortality observed in any of the species tested.

There were slight phytotoxic effects observed in oilseed rape, soybean, tomato, sugar beet, and perennial ryegrass. However, these were not statistically significant at any test concentration for any species as compared to the control. There were no phytotoxic effects observed in the test species onion.

The ER_{50} values were determined to be >5.00, 4.22, >5.00, >5.00, >5.00 and >5.00 L test item/ha for oilseed rape, soybean, tomato, sugar beet, perennial ryegrass and onion, respectively.

I. Materials and Methods

Materials

Test Material	Prothioconazole + Spiroxamine EC 460
Lot/Batch #:	EM4L025614
Purity:	Prothioconazole: 16.2% w/w (159.0 g/L) Spiroxamine: 30.1% w/w (295.7 g/L)
Description:	Yellow-brown liquid
Reanalysis/Expiry date:	08 April 2021
Density:	0.983 g/mL
Treatments	
Test rates:	Nominal: 0.31, 0.63, 1.25, 2.50 and 5.00 L test item/ha
Test organisms	
Species:	Perennial ryegrass (*Lolium perenne*), onion (*Allium cepa*), oilseed rape (*Brassica napus*), soybean (*Glycine max*), tomato (*Solanum lycopersicum*), sugar beet (*Beta vulgaris*)
Source:	Not reported
Test design	
Test vessel:	Commercial plastic flower pots, 15 cm diameter
Test medium:	Sterilised soil at pH 5.9 ± 0.5; C_{org} 0.66 ± 0.07%
Replication:	Five – ten pots per treatment group
No. /vessel:	At least 20 plants per treatment group
Duration of test:	21 days
Environmental test conditions	
Temperature:	22°C ± 10°C
Relative humidity:	70% ± 25%
pH:	5.9 ± 0.5
Photoperiod:	16 hours light, 8 hours dark (light intensity minimum 200 µE/m²/s)

Study Design

This study was conducted in order to evaluate the effect of Prothioconazole + Spiroxamine EC 460 on the vegetative vigour of monocot and dicot crops.

Test species were monocotyledonous plants from two families (perennial ryegrass and onion) and dicotyledonous plants from four different families (oilseed rape, soybean, tomato and sugar beet).

Plants were grown in commercial plastic flower pot in a growth chamber at 22 ± 10 °C under a 16 hour light 8 hour dark photoperiod. Between five and ten replicates with twenty seeds per species were tested.

Sterilised sandy loam soil was used as the test medium.

At test initiation, spray solution made up of the test item dissolved in deionised water was applied to the soil surface using a spray chamber with an overhead nozzle (set at 40 cm above sprayed surface). The test item was sprayed on the leaves and above-ground portions of plant.

Observations of phytotoxicity were made on days 7, 14 and 21 according to the EPPO Standard 155. Fresh weights were determined at the final assessment. Observations of mortality were made on days 7, 14 and 21 after application. Growth stages at test initiation and test termination were recorded according to BBCH-Monograph – Growth stages.

Statistical analysis was carried out on the fresh weight data using the Shapiro-Wilk's test ($\alpha = 0.05$) and the Levene's test ($\alpha = 0.05$). The Dunnett's t-test (multiple comparison, one-sided smaller, $\alpha = 0.05$) was used if the data were normally distributed and homogeneous. The Williams t-test (multiple comparison, one sided-smaller, $\alpha = 0.05$) was used if the data showed a monotonic dose response. If the data were not homogeneous the Bonferroni-Welch t-test (multiple comparison, one-sided smaller, $\alpha = 0.05$) was used.

Probit analysis was used to determine the EC_{10}, EC_{20} and EC_{50} values.

The software used to perform the statistical analysis was ToxRat Professional, Version 3.3.0, ® ToxRat Solutions GmbH.

Analytical method

Samples of water were analysed using the validated analytical method M-688131-01-1, report reference M-688131-01-1 **(see Doc MCP Section 5)**.

II. Results and Discussion

Validity criteria according to the OECD 227 guideline (2006) were met.

- The seedling emergence is at least 70% (actual: 89-97%)
- The plants do not exhibit visible phytotoxic effects. Plants exhibit only normal variation in growth and morphology for that particular species (actual: achieved)
- The mean plant survival is at least 90% for the duration of the study (actual: 100%)
- Environmental conditions for a particular species are identical and growing media contain the same amount of soil matrix, support media, or substrate from the same source (actual: achieved)

The analytical recovery rate of the active substance prothioconazole in the stock solution was 108 % of the nominal value and 104 % of the nominal value for spiroxamine, respectively.

There were some statistically significant reductions in the observed fresh weight of oilseed rape, soybean and tomato which were significantly reduced at 1.25, 2.50 and 5.00 L test item/ha and sugar beet, which was reduced at 2.50 and 5.00 L test item/ha. However, there were no statistically significant reductions in the observed fresh weight of perennial ryegrass and onion.

Table CP 10.6.2/04-1 Summarised results of fresh weight and plant growth

Species	Treatment group (L test item/ha)	Fresh weight (g) 21 days	Standard deviation	Effect[1] (%)	Growth stage (BBCH) at application	Growth stage (BBCH) 21 days
Oilseed rape	Control	41.79	± 4.47	-	12-13	16-18
	0.31	43.13	± 2.74	3.2	12-13	16-18
	0.63	41.91	± 3.42	0.3	12-13	16-18

Species	Treatment group (L test item/ha)	Fresh weight (g) 21 days	Standard deviation	Effect[1] (%)	Growth stage (BBCH) at application	Growth stage (BBCH) 21 days
	1.25	34.20	± 5.57	-18.2*	12-13	16-18
	2.50	32.58	± 2.69	-22.0*	12-13	16-18
	5.00	23.17	± 6.66	-44.6*	12-13	16-18
Soybean	Control	28.17	± 2.25	-	12	61
	0.31	28.41	± 2.68	0.8	12	61
	0.63	27.59	± 3.17	-2.1	12	61
	1.25	24.57	± 3.06	-12.8*	12	61
	2.50	20.91	± 3.12	-25.8*	12	61
	5.00	11.70	± 3.67	-58.5*	12	13-16
Tomato	Control	31.20	± 3.46	-	12	15
	0.31	25.37	± 7.84	-18.7	12	15-16
	0.63	23.71	± 9.70	-24.0	12	15-16
	1.25	25.42	± 9.48	-18.5*	12	15-16
	2.50	21.31	± 3.36	-31.7*	12	15
	5.00	20.78	± 6.28	-33.4*	12	15
Sugar beet	Control	21.22	± 3.80	-	12	14-16
	0.31	21.47	± 3.92	1.2	12	14-16
	0.63	21.12	± 3.42	-0.5	12	14-16
	1.25	19.67	± 3.14	-7.3	12	14-16
	2.50	17.01	± 2.65	-19.8*	12	14-16
	5.00	14.75	± 3.69	-30.5*	12	14-16
Perennial ryegrass	Control	14.75	± 1.81	-	12-13	22-23
	0.31	13.91	± 1.95	-5.7	12-13	22-23
	0.63	13.12	± 2.31	-11.1	12-13	22-23
	1.25	14.23	± 1.94	-3.5	12-13	22-23
	2.50	14.62	± 1.94	-0.9	12-13	22-23
	5.00	13.82	± 1.14	-6.3	12-13	22-23
Onion	Control	11.39	± 2.53	-	12	14-15
	0.31	12.56	± 1.41	10.2	12	14-15
	0.63	11.67	± 1.55	2.5	12	14-15
	1.25	12.19	± 1.68	7.0	12	14-15
	2.50	11.73	± 1.94	3.0	12	14-15
	5.00	11.68	± 2.14	2.5	12	14-15

[1] negative values indicate reduction compared to the control

* statistically significant difference (α=0.05)

There was no mortality observed in any test species at any test concentration. Phytotoxic effects were observed in oilseed rape, soybean, tomato, sugar beet, and perennial ryegrass. Phytotoxic effects were most pronounced at the highest treatment level of 5.00 L test item/ha. There were no phytotoxic effects observed in the test species onion.

Table CP 10.6.2/04-2 Summarised results of mortality and phytotoxicity

Species	Treatment group (L test item/ha)	Mortality (%)	Phytotoxicity (%)		
			7 days	14 days	21 days
Oilseed rape	Control	0	0	0	0
	0.31	0	0	0	0
	0.63	0	2	2	2
	1.25	0	6	11	16
	2.50	0	22	26	17
	5.00	0	49	49	37
Soybean	Control	0	0	0	0
	0.31	0	2	2	2
	0.63	0	9	6	3
	1.25	0	14	8	8
	2.50	0	26	15	19
	5.00	0	51	47	60
Tomato	Control	0	0	0	0
	0.31	0	0	1	1
	0.63	0	0	1	2
	1.25	0	2	9	5
	2.50	0	12	12	6
	5.00	0	34	24	15
Sugar beet	Control	0	0	0	0
	0.31	0	0	0	0
	0.63	0	4	3	2
	1.25	0	4	4	4
	2.50	0	10	15	13
	5.00	0	16	39	38
Perennial ryegrass	Control	0	0	0	0
	0.31	0	0	0	0
	0.63	0	0	0	0
	1.25	0	0	0	0
	2.50	0	0	0	0
	5.00	0	4	4	2
Onion	Control	0	0	0	0
	0.31	0	0	0	0
	0.63	0	0	0	0
	1.25	0	0	0	0
	2.50	0	0	0	0
	5.00	0	0	0	0

Table CP 10.6.2/04-1 Summary of endpoints

Species	NOER	LOER		ER_{10}	ER_{20}	ER_{50}
	(L test item/ha)			(L test item/ha)		
Oilseed rape	0.63[3]	1.25[3]		1.09[4]	1.97	>5.00[4]
			Lower 95% CL	0.135	0.685	4.05
			Upper 95% CL	1.82	2.89	>5.00
Soybean	0.63[3]	1.25[3]		1.27[4]	1.92[4]	4.22[4]
			Lower 95% CL	0.830	1.46	3.66
			Upper 95% CL	1.63	2.29	>5.00
Tomato	0.63[1]	1.25[1]		>5.00	>5.00	>5.00
			Lower 95% CL	n.d.	n.d.	n.d.
			Upper 95% CL	n.d.	n.d.	n.d.
Sugar beet	1.25[2]	2.50[2]		1.54[4]	2.93[4]	>5.00[4]
			Lower 95% CL	0.798	2.18	>5.00
			Upper 95% CL	2.09	3.68	>5.00
Perennial ryegrass	≥5.00[3]	>5.00		>5.00	>5.00	>5.00
			Lower 95% CL	n.d.	n.d.	n.d.
			Upper 95% CL	n.d.	n.d.	n.d.
Onion	≥5.00[3]	>5.00		>5.00	>5.00	>5.00
			Lower 95% CL	n.d.	n.d.	n.d.
			Upper 95% CL	n.d.	n.d.	n.d.

[1] multiple comparison Bonferroni-Welch t-test, $\alpha = 0.05$
[2] multiple comparison Williams t-test, $\alpha = 0.05$
[3] multiple comparison Dunnett's t-test, $\alpha = 0.05$
[4] Probit Analysis, CL = confidence limits

III. Conclusion

The most sensitive species in terms of fresh weight was soybean with an EC_{50} value of 4.22 L test item/ha (EC_{20} value of 1.92 L test item/ha).

Then followed oilseed rape and sugar beet, both with EC_{50} values of >5.00 L test item/ha (EC_{20} values of 1.97 and 2.93 L test item/ha, respectively).

For tomato, an EC_{50} value could not be determined because of a lacking dose-response relationship. The NOEC of this species was 0.63 L test item/ha. However, as the effects at all test concentrations were below 50%, the EC_{50} value for this species is considered to be >5.00 L test item/ha.

The fresh weight of onion and perennial ryegrass was not affected up to 5.00 L test item/ha, thus the EC_{50} value for these two species was considered to be >5.00 L test item/ha.

No mortality was observed for any species tested.

Phytotoxic effects observed were chlorosis, necrosis, growth reduction and leaf and stem deformations on the dicotyledonous species. For onion, no phytotoxic effects were observed.

Assessment and conclusion by applicant:

This is a new study that has not been previously evaluated or submitted.

Validity criteria according to the OECD 227 guideline (2006), to which the study was conducted, were met.

- The seedling emergence is at least 70% (actual: 89-97%)
- The plants do not exhibit visible phytotoxic effects. Plants exhibit only normal variation in growth and morphology for that particular species (actual: achieved)
- The mean plant survival is at least 90% for the duration of the study (actual: 100%)
- Environmental conditions for a particular species are identical and growing media contain the same amount of soil matrix, support media, or substrate from the same source (actual: achieved)

Analytical verification of the stock solution used for the spray solutions confirmed correct dosing of the test system. The study is therefore considered acceptable.

The most sensitive species in terms of fresh weight was soybean with an EC_{50} value of 4.22 L test item/ha.

Data Point:	KCP 10.6.2/01
Report Author:	[redacted]
Report Year:	2007
Report Title:	Non-target terrestrial plants: An evaluation of the effects of JAU 6476 & KWG 4168 EC 160 + 300 in the seedling emergence and growth test
Report No:	SE04/07
Document No:	M-182345-02-1
Guideline(s) followed in study:	OECD 208 A (July 2000, draft): seedling emergence and growth test (Tier 1)
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and accepted RAR (2010)
GLP/Officially recognised testing facilities:	No, not conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Supportive only

Executive Summary

The effect of JAU 6476 & KWG 4168 EC 160 + 300 on the seedling emergence of monocot (corn, *Zeya mays*; oats, *Avena sativa*) and dicot (cucumber, *Cucumis sativus;* oilseed rape, *Brassica napus*; soybean, *Glycine max*; sunflower, *Helianthus annuus L.*) crops was studied at a single treatment rate of 1.25 L product/ha.

The growth medium used in the test was sterilised soil (pH: 7.4; organic carbon: 1.19%).

All seeds were planted on the day of test item application and the test duration was 14 days after 65% emergence of the seedlings in the controls for each species. Spray treatments were once applied, at test initiation, using a spray volume of 200 litres/ha.

The percentage decrease in germination was 5%, 5%, 0%, 11%, 14% and 15% in corn, oats, cucumber, oilseed rape, soybean and sunflower respectively. Biomass was reduced in oilseed rape by 14% and was increased in corn, oats, cucumber, soybean and sunflower by 2%, 12%, 11%, 1% and 19% respectively. Phtotoxicity was not observed in any species tested. No differences either reached or exceeded the 50% trigger for further testing or were significant at the 95% confidence limits.

The ER_{50} was considered to be >1.25 L product/ha for all species tested.

I. Materials and Methods

Materials

Test Material	JAU 6476 & KWG 4168 EC 160 + 300
Lot/Batch #:	06920/0084(0079)
Purity:	Not reported
Description:	Clear brown liquid
Stability of test compound:	Not reported
Reanalysis/Expiry date:	28 January 2006
Density:	0.985 g/mL
Treatments	
Test rates:	Nominal: 1.25 L product/ha
Solvent/vehicle:	None reported
Test organisms	
Species:	Corn (*Zeya mays*), oats (*Avena sativa*), cucumber (*Cucumis sativus*), oilseed rape (*Brassica napus*), soybean (*Glycine max*) and sunflower (*Helianthus annuus L*)
Source:	Seeds supplied from commercial sources via Bayer CropScience GmbH, Horticulture, H872, 65926 Frankfurt am Main
Test design	
Test vessel:	Commercial plastic flower pots, 10-cm diameter
Test soil:	Sterilised soil, pH 7.4, C_{org} 1.19%
Replication:	Four pots per treatment group
No. /vessel:	Five seeds per test vessel
Duration of test:	14 days after 65 % emergence of the seedling in the controls for each species
Environmental test conditions	
Temperature:	23 ± 5 °C day, 18 ± 5 °C night
Photoperiod:	16 hrs light, 8 hrs dark. Natural daylight supplemented by artificial lighting to provide the required photoperiod > 10000 lux lamps turn off, > 20000 lux shading closing.

Study Design

This study was conducted in order to evaluate the effect of JAU 6476 & KWG 4168 EC 160 + 300 on the seedling emergence of monocot and dicot crops.

Test species were monocotyledonous plants from one family (corn and oats) and dicotyledonous plants from four different families (cucumber, oilseed rape, soybean and sunflower).

Plants were grown in commercial plastic flower pot in a glasshouse at 23 ± 5 °C during the day and 18 ± 5°C at night under a 16 hour light 8 hour dark photoperiod. Four replicates with five seeds per pot for each species were tested.

Test soil was sterilised with 120 degrees vapour for approximately 30 minutes and fertilised with 2.4 g Blaukorn per L. Soil was composed of 14.2% sand, 65.1% silt and 20.7% clay.

At test initiation, spray solution made up of the test item dissolved in deionised water was applied to the soil surface using a spray chamber with an overhead nozzle (set at 50 cm above sprayed surface).

Observations of phytotoxicity were made on days 7 and 14 (after the emergence of 65% seedling emergence). Growth stages were recorded at the final assessment according to the BBCH-Monograph Growth stages. Dry weights were determined at the final assessment. Dead plants were removed after each assessment (mortality following application was recorded at the final assessment).

Statistical analysis was carried out using the Pairwise Mann-Whitney-U-test to determine significant differences between control and treatment for any species at the 95% confidence limit.

II. Results and Discussion

Validity criteria were not assessed as part of the study report.

No marked or statistically significant effects of treatment on the germination rate were observed in any species.

Mortality occurred in two untreated soybean plants and one plant with cucumber, oilseed rape and soybean treatment.

There were no visual phytotoxicity nor differences in growth stages in any species.

Table CP 10.6.2/01-1 Germination rate after exposure to JAU 6476 & KWG 4168 EC 160 + 300

Crop	Control		1.25 L product/ha	
	Number	% of sown	Number	% of sown
Corn	20	100	19	95
Oats	20	100	19	95
Cucumber	19	95	20	100
Oilseed rape	19	95	18	90
Soybean	16	80	13	65
Sunflower	20	100	17	85

Table CP 10.6.2/01-2 Survival and mortality after exposure to JAU 6476 & KWG 4168 EC 160 + 300

Crop	Control		1.25 L product/ha	
	Number	% mortality	Number	% mortality
Corn	0	0	0	0
Oats	0	0	0	0
Cucumber	0	0	1	5
Oilseed rape	0	0	1	6
Soybean	2	13	1	8

Crop	Control		1.25 L product/ha	
	Number	% mortality	Number	% mortality
Sunflower	0	0	0	0

Table CP 10.6.2/01-3 Phytotoxicity and growth stage after exposure to JAU 6476 & KWG 4168 EC 160 + 300

Crop	Phytotoxicity		Growth stage	
	Control	1.25 L product/ha (% of control)	Control	1.25 L product/ha (% of control)
Corn	0	0	13	13
Oats	0	0	13	13
Cucumber	0	0	12	12
Oilseed rape	0	0	14	14
Soybean	0	0	13-14	13-14
Sunflower	0	0	16	16

Table CP 10.6.2/01-4 Dry weight after exposure to JAU 6476 & KWG 4168 EC 160 + 300

Species	Treatment	Pot		Plant		
		Mean dry weight (g)	Mean no. of plants on day 21	Mean dry weight (g)	SD	Deviation from control (%)
Corn	Control	1.085	5	0.217	0.031	
	Treated	1.055	4.75	0.220	0.029	+2
Oats	Control	0.512	5	0.104	0.009	
	Treated	0.542	4.75	0.116	0.029	+12
Cucumber	Control	1.312	5.5	0.256	0.022	
	Treated	1.342	4.75	0.284	0.020	+11
Oilseed rape	Control	1.223	4.75	0.256	0.024	
	Treated	0.926	4.25	0.221	0.041	-14
Soybean	Control	1.462	3.5	0.418	0.014	
	Treated	0.753	3	0.422	0.177	+1
Sunflower	Control	0.839	5	0.166	0.013	
	Treated	0.820	4.25	0.198	0.038	+19

SD Standard deviation

III. Conclusion

The highest nominal product application rate of 1.25 L product/ha JAU 6476 & KWG 4168 EC 160 + 300 showed no significant adverse effect (*i.e* greater than 50%) to representative non-target crops in the seedling emergence and growth test. The ER_{50} was considered to be >1.25 L product/ha for all species tested.

Assessment and conclusion by applicant:

This non-GLP study was previously evaluated and accepted.

Validity criteria according to the current OECD 208 test guideline (2000) have been assessed.

- The seedling emergence is at least 70% (actual: 80 to 100%)
- The seedlings do not exhibit visible phytotoxic effects (*e.g.* chlorosis, necrosis, wilting, leaf and stem deformations) and the plants exhibit only normal variation in growth and morphology for that particular species (actual: there were no visual phytotoxicity nor differences in growth stages in any species).
- Environmental conditions for a particular species are identical and growing media contain the same amount of soil matrix, support media, or substrate from the same source

However, some validity criteria were not fully complied with:

- The mean survival of emerged control seedlings is at least 90% for the duration of the study (actual: 100% survival except soybean which had 87% survival)

The criterion for survival was not met for soybean but it is noted that this was only slightly below the threshold of 90%. The results are considered to be valid but as the data were not generated under GLP, the study has been submitted as supporting information only. It should also be noted that the results are consistent with the results of the more recent GLP study, M-688131-01-1.

The ER_{50} was considered to be >1.25 L product/ha for all species tested.

Data Point:	KCP 10.6.2.02
Report Author:	[redacted]
Report Year:	2007
Report Title:	Non-target terrestrial plants: An evaluation of the effects of JAU 6476 & KWG 4168 EC 160 + 300 in the vegetative vigour test
Report No:	VV04/07
Document No:	M-182346-02-2
Guideline(s) followed in study:	OECD 208 A (July 2000, draft): vegetative vigour test (Tier 1)
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and accepted RAR (2010)
GLP/Officially recognised testing facilities:	No, not conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Supportive only

Executive Summary

The effect of JAU 6476 & KGW 4168 EC 160 + 300 on the vegetative vigour of monocot (corn, *Zea mays*; oats, *Avena sativa*) and dicot (cucumber, *Cucumis sativus*; oilseed rape, *Brassica napus*; soybean, *Glycine max*; lettuce, *Lactuca sativa*) crops was studied at 1.25 L product/ha (the highest nominal

product application rate for JAU 6476 & KGW 4168 EC 160 + 300). The growth medium used in the test was sterilised soil (pH: 7.4; organic carbon: 1.19%). Plants were treated with foliar spray application at the 2-4 leaf stage.

Spray treatments were applied once, at test initiation, at the nominal spray volume of 200 litres/ha. Plants were assessed for mortality and phytotoxicity on days 7, 14 and 21. At study termination, endpoint determinations were performed for plant dry weights.

There was no adverse effect of JAU 6476 & KWG 4168 EC 160 + 300 on the mortality of the six species tested. Phytotoxicity was observed as necrosis and leaf deformation in cucumber, oilseed rape, soybean and lettuce which were rated at 15%.

Biomass was reduced in corn, oats, oilseed rape and soybean by 12%, 5%, 16% and 20% respectively. Biomass was reduced in cucumber and lettuce by 3% and 12% respectively. No differences either reached or exceeded the 50% trigger for further testing or were significant at the 95% confidence limits. The ER_{50} was considered to be >1.25 L product/ha for all species tested.

I. Materials and Methods

Materials

Test Material	JAU 6476 & KGW 4168 EC 160 + 300
Lot/Batch #:	06920/0084(0079)
Purity:	150.60 g/L JAU 6476; 296.49 g/L KWG 4168
Description:	Clear brown liquid
Stability of test compound:	Not reported
Reanalysis/Expiry date:	28 Jan 2005
Density:	0.985 g/mL
Treatments	
Test rates:	1.25 L product/ha
Test organisms	
Species:	Corn (*Zea mays*), oats (*Avena sativa*), cucumber (*Cucumis sativus*), oilseed rape (*Brassica napus*), soybean (*Glycine max*) and lettuce (*Lactuca sativa*)
Source:	Seeds supplied from commercial sources via Bayer CropScience GmbH, Horticulture, H 872, 65926 Frankfurt am Main
Test design	
Test vessel:	Commercial plastic flower pots, 10 and 13 cm diameter
Test soil:	Sterilised soil at pH 7.4; C_{org} 1.19%
Replication:	Four pots per treatment group
No. animals/vessel:	Five plants per pot
Duration of test:	21 days
Environmental test conditions	
Temperature:	23 ± 5 °C day, 18 ± 5 °C night

Photoperiod: 16 hrs light, 8 hrs dark. Natural daylight supplemented by artificial lighting to provide the required photoperiod > 10000 lux lamps turn off, > 20000 lux shades close

Study Design

This study was conducted in order to evaluate the effect of JAU 6476 & KWG 4168 EC 160 + 300 on the vegetative vigour of monocot and dicot crops.

Test species were monocotyledonous plants from one family (corn and oats) and dicotyledonous plants from four different families (cucumber, oilseed rape, soybean and lettuce).

Plants were grown in commercial plastic flower pot in a glasshouse at 23 ± 5 °C during the day and 18 ± 5 °C at night under a 16 hour light 8 hour dark photoperiod. Four replicates with five plants per pot for each species were tested.

Test soil was sterilised with 120 degrees vapour for approx. 30 minutes and fertilised with 2.4 g Blaukorn per L. Soil was composed of 14.2% sand, 65.1% silt and 20.7% clay.

At test initiation, spray solution made up of the test item dissolved in deionised water was applied to the soil surface using a spray chamber with an overhead nozzle (set at 50 cm above sprayed surface). The test item was sprayed on the leaves and above-ground portions of plant.

Observations of phytotoxicity were made on days 7, 14 and 21 according to the EPPO Standard 135. Dry weights were determined at the final assessment. Dead plants were removed after each assessment (mortality following application was recorded at the final assessment).

Statistical analysis was carried out using the Pairwise Mann-Whitney-U test to determine significant differences between control and treatment for any species at the 95% confidence limit.

II. Results and Discussion

Validity criteria were not assessed as part of the study report.

No mortality was observed in any of the species shown throughout the test.

Phytotoxic symptoms visualised as necrosis and leaf deformation were observed at test end for 1.25 L product/ha treatment.

There were no effects on growth stage development of treated plants in comparison to the untreated controls at all application rates tested.

Biomass was reduced in corn, oats, oilseed rape and soybean by 12%, 5%, 16% and 20%, respectively. Biomass was increased in cucumber and lettuce by 3% and 12%, respectively. However, none of these differences were significant at the 95% confidence limits and none of the differences reached or exceeded the 50% trigger for further testing.

Table CP 10.6.2/02-1 Survival and mortality after exposure to JAU 6476 & KWG 4168 EC 160 + 300

Crop	Control		1.25 L product/ha	
	Number	% mortality	Number	% mortality
Corn	20	0	20	0
Oats	20	0	20	0
Cucumber	20	0	20	0
Oilseed rape	20	0	20	0
Soybean	20	0	20	0

Crop	Control		1.25 L product/ha	
	Number	% mortality	Number	% mortality
Lettuce	20	0	20	0

Table CP 10.6.2/02-2 Phytotoxicity and growth stage after exposure to JAU 6476 & KWG 4168 EC 160 + 300

Crop	Phytotoxicity		BBCH Growth stage	
	Control	1.25 L product/ha (% of control)	Control	1.25 L product/ha (% of control)
Corn	0	0	16-17	16-17
Oats	0	0	51	51
Cucumber	0	15	51	51
Oilseed rape	0	15	51-65	51-59
Soybean	0	15	71	71
Lettuce	0	15	24-28	24-28

Table CP 10.6.2/02-3 Dry weight after exposure to JAU 6476 & KWG 4168 EC 160 + 300

Plant	Treatment	Pot		Plant	
		Mean dry weight (g)	Total survival	Mean dry weight (g)	Deviation from control (%)
Corn	Control	23.91	20	4.782	
	Treated	21.06	20	4.211	-12
Oats	Control	13.86	20	2.772	
	Treated	13.18	20	2.636	-5
Cucumber	Control	12.47	20	3.142	
	Treated	13.00	20	3.250	+3
Oilseed rape	Control	16.74	20	3.348	
	Treated	14.13	20	2.825	-16
Soybean	Control	12.53	20	2.505	
	Treated	10.01	20	2.002	-20
Lettuce	Control	7.60	20	1.521	
	Treated	8.50	20	1.700	+12

III. Conclusion

The highest nominal product application rate of 1.25 L product/ha JAU 6476 & KWG 4168 EC 160 + 300 showed no significant adverse effect (*i.e* greater than 50%) to representative non-target crops in the vegetative vigour test. The ER_{50} was considered to be >1.25 L product/ha for all species tested.

Assessment and conclusion by applicant:

This study was previously submitted and accepted.

Validity criteria according to the OECD 227 (2006) guideline were assessed:

- Control plant survival ≥90% (actual: 100% all species)
- Control plants to not exhibit visible phytotoxic effects (actual: achieved)
- Environmental conditions for a particular species are identical and growing media contain the same amount of soil matrix, support media or substrate from the same source (actual: achieved)

The validity criterion of at least 70% seedling emergence of plants used for the test could not be verified from the information available, however this deviation is not thought to affect the validity of the study as the survival of all control plants was 100%.

The results are considered to be valid but as the data were not generated under GLP, the study has been submitted as supporting information only. It should also be noted that the results are consistent with the results of the more recent GLP study, M-688315-01-1.

The ER_{50} was considered to be >1.25 L product/ha for all species tested.

CP 10.6.3 Extended laboratory studies on non-target plants

No data for extended laboratory studies with non-target terrestrial plants are available. These data are not necessary as an acceptable risk has been demonstrated for the proposed use of Prothioconazole + Spiroxamine EC 460 using the available Tier I laboratory data.

CP 10.6.4 Semi-field and field tests on non-target plants

No data for semi-field or field studies with non-target terrestrial plants are available. These data are not necessary as an acceptable risk has been demonstrated for the proposed use of Prothioconazole + Spiroxamine EC 460 using the available Tier I laboratory data.

CP 10.7 Effects on other terrestrial organisms (flora and fauna)

All required and available data have been submitted and evaluated in the presented risk assessments. No further data are available or thought to be necessary with other terrestrial organisms.

CP 10.8 Monitoring data

Monitoring of exposure of non-target flora and fauna to spiroxamine has not been conducted. The risk assessments presented in this document demonstrate that there are no unacceptable risks to the environment and non-target organisms when spiroxamine is applied in accordance with the proposed use of Prothioconazole + Spiroxamine EC 460.